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The Consequences of Plant Species Diversity and Genetic Diversity for Populations, Communities, and Ecosystems

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ABSTRACT

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Plant species diversity plays an important role in structuring communities and mediating ecosystem processes. Experiments have shown increasing plant species richness increases primary productivity, arthropod species richness, nutrient cycling, and community stability. Because effects of diversity are driven by variation among individuals, it is expected that genetic diversity within a species may mimic the effects of species diversity. Indeed, recent experimental evidence has confirmed this prediction. However, few studies have simultaneously investigated the effects of plant species diversity and genetic diversity in the same system. Therefore, the relative importance of species diversity and genetic diversity for community structure and ecosystem processes remains unresolved, and, importantly, potential interactions between levels of diversity have rarely been investigated. Interactive effects between genetic diversity and species diversity are particularly important to investigate, as natural systems are composed of several genotypes of many different species. Here, I investigated how plant species diversity and genetic diversity influenced populations, communities, and ecosystems. First, I tested whether genetic diversity within populations of a weedy annual plant, *Arabidopsis thaliana*, influenced population success. Increasing genetic diversity increased several measures of population viability, including seedling emergence, biomass production, flowering duration, and seed set. This result suggests that highly

genetically diverse populations, such as populations created from multiple introductions, may be more able to colonize novel environments than less genetically diverse populations. Next, I simultaneously manipulated plant species diversity and genetic diversity within a dominant plant species in a common garden. This experiment addressed how both levels of diversity influenced a key ecosystem process, primary productivity. Plant species diversity and genetic diversity interactively influenced biomass production, with productivity increasing the most with genetic diversity when high levels of species diversity were present. Finally, I explored how plant diversity in the common garden influenced arthropod community composition, and found that genetic diversity influenced arthropods more strongly than plant species diversity. Altogether, my work underscores the importance of understanding how plant species diversity and genetic diversity interactively influence ecological communities in order to gain a more holistic view of how communities are structured and what factors control ecosystem functioning.

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Chapter 1

Population Genetic Diversity Influences Colonization Success

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Much thought has been given to the individual-level traits that may make a species a successful colonizer. However, these traits have proven to be imperfect predictors of colonization success. Here, we test whether population-level characteristics, specifically genetic diversity and density, can influence colonization ability on a short-term timescale, independent of longer-term effects on adaptive potential. Within experimentally manipulated populations of the weedy herb *Arabidopsis thaliana*, we found that increased genetic diversity increased colonization success measured as population-level seedling emergence rates, biomass production, flowering duration, and reproduction. Additive and non-additive effects contributed to these responses, suggesting that both individual genotypes (sampling effect) and positive interactions among genotypes (complementarity) contributed to increased colonization success. In contrast, manipulation of plant density had no effect on colonization success. The heightened ability of relatively genetically rich populations to colonize novel habitats, if a general phenomenon, has important implications for predicting and controlling biological invasions. Specifically, cases where multiple introductions of a species are likely could lead to an increased probability of invasion.

1.1 INTRODUCTION

The colonization of novel habitats plays an important role in many ecological processes, from ecological succession to population dynamics and range expansion. Colonization requires species to both disperse to a novel environment and successfully survive and reproduce there. While much thought has been given to the individual-level traits that may make a species a successful disperser (Rees *et al.* 2001), far less attention has been paid to population-level characteristics (e.g., density, age structure, genetic variation) that may affect the probability of survival and expansion of the colonizing population. Traditionally, individual-level traits such as dispersal ability, generation time, and growth rate have received a great deal of consideration as predictors of colonization ability (for animals, see Sol 2007; for plants, see Baker 1974, Mack *et al.* 2000, Kolar & Lodge 2001, Sakai *et al.* 2001, Rejmánek *et al.* 2005, Pyšek & Richardson 2007, Whitney & Gabler 2008). However, these traits are weak predictors (Perrins *et al.* 1992, Mack 1996), and characteristics arising at the population or species level (such as genetic diversity and population density) may offer greater explanatory power.

Variation in the genetic diversity of founder populations is extensive (reviewed in Novak & Mack 2005, Roman & Darling 2007, Dlugosch & Parker 2008) and has the potential to influence the success of colonization events. Many recent studies have found that populations of species colonizing novel habitats harbor genetic diversity as high as or higher than that found in populations in their native range (e.g., Kolbe *et al.* 2004, Genton *et al.* 2005). Sources of such high levels of diversity include admixture (defined here as the mixture of individuals from geographically and genetically distinct source populations) and gene flow among multiple independent introductions of the species. For

example, multiple introductions of agriculturally important genotypes of reed canarygrass, *Phalaris arundinacea*, have lead to higher within-population genetic diversity in parts of the grass's non-native range than in its native range (Lavergne & Molofsky 2007). Even in cases of small founder populations not subject to human-mediated dispersal, admixture can occur at high levels. During colonization of the small Galapagos island of Daphne Major by large ground finches *Geospiza magnirostris*, small immigrant populations ($\leq$ 39 genotyped individuals) were derived from as many as five genetically distinct source islands (Grant *et al.* 2001). Nevertheless, some colonization events may only involve a few individuals that represent a small fraction of the natural genotypic variation of the species, effectively creating a population bottleneck (e.g., Kliber & Eckert 2005, Puillandre *et al.* 2008).

Much work has focused on the long-term evolutionary consequences of bottlenecks and/or multiple introductions for the adaptive potential of founder populations; however, relatively little attention has been paid to the short-term effects of genetic diversity on colonization success. Colonizing species experience habitats that often differ in abiotic or biotic conditions relative to their native ranges, and genetic variation within these populations is expected to increase colonization success in novel conditions by allowing rapid adaptation (Sakai *et al.* 2001, Lee 2002, Holt *et al.* 2005). In contrast, short-term effects (traditionally termed "ecological" effects, although clearly evolution can happen rapidly enough to conflate ecological and evolutionary time scales; see Antonovics 1976, Hairston *et al.* 2005) conceivably could play out within the first generation. For example, high levels of genetic diversity could help a population become established, either by increasing the odds that some individuals can withstand the novel

conditions (lottery model or sampling effect), or by allowing more efficient or more complete use of resources (niche partitioning) (Huston 1997, Loreau & Hector 2001). Studies of species in their native habitats are consistent with this view (Hughes *et al.* 2008). For example, populations of *Clarkia pulchella* with relatively high levels of genetic diversity maintained larger population sizes over the course of four years than less genetically diverse populations (Newman & Pilson 1997). Within a single generation, increased genetic diversity increased population biomass in goldenrod (*Solidago*) (Crutsinger *et al.* 2006) and population resistance and resilience to disturbance in eelgrass (*Zostra*) (Hughes & Stachowicz 2004, Reusch *et al.* 2005). Additionally, higher levels of population-level genetic diversity in a barnacle species increased larval settling success (Gamfeldt *et al.* 2005), although post-settling performance (growth, reproduction) was not measured. Thus, the available data indicate that increased genetic diversity could translate into higher population growth rates and higher initial dispersal to habitats for colonizing species.

However, if our goal is to examine how increased genetic variation in a founding population influences colonization success, the aforementioned experiments are not ideal, as they utilized genotypes that evolved in a common environment in their native ranges and were designed to address different questions. A more direct test of the hypothesis would examine population performance in an introduced or novel environment and create diversity treatments from divergent genotypes from multiple source locales, mimicking the admixture process.

To examine how genetic diversity may affect a species' ability to colonize a novel environment, we chose to use the model organism *Arabidopsis thaliana* (Brassicaceae).

Arabidopsis thaliana has been widely used in molecular biology studies, resulting in the accessibility of numerous ecotypes with well-characterized genetic and phenotypic variation (Mitchell-Olds 2001, Pigliucci 2002). It also is a widespread weed that has colonized numerous habitats on four continents (Clarke 1993) and thus provides an excellent model system for examining questions at the interface of genetics, ecology, and invasion biology (Weltzin *et al.* 2003). By manipulating genetic diversity within populations of *A. thaliana* and measuring colonization success, we focus attention on whether high genetic diversity can promote invasion success on a short-term, "ecological" timescale. We used soil-filled trays in a greenhouse as the novel environment to be colonized. While this approach clearly lacks the realism of the field, it does increase the conservatism of the test for effects of genetic diversity (e.g., the more uniform soil and climatic conditions should decrease opportunities for complementarity in resource use, relative to the field). We also manipulated plant density. We included density as a factor because it should influence the degree of interaction between individual plants, and we hypothesized that such interactions among genotypes may be an important mechanism influencing colonization success. Specifically, we address the question: Does greater population-level genetic diversity and/or density increase the ability of a species to initially colonize a novel environment via enhanced survivorship, growth, and/or reproduction?

1.2 METHODS

Plant material -- *Arabidopsis thaliana* is a predominantly selfing, weedy herb with a hypothesized origin in Eurasia (Mitchell-Olds 2001, Pigliucci 2002). Twenty-three ecotypes were obtained from the Arabidopsis Biological Resource Center housed at

Ohio State University. Stocks of each of these ecotypes have generally been bred from a single seed and maintained as an inbred line (Arabidopsis Biological Resource Center, Columbus, Ohio), so we consider each ecotype to represent a single genotype. We chose accessions (Table 1.1) that maximized microsatellite marker diversity (King *et al.* 1993, Innan *et al.* 1997, Kover & Schaal 2002) and also represented a broad swath of the species range. During the summer of 2006, *Arabidopsis* plants were reared from seed in a common growth chamber for bulk seed production and to reduce potential maternal environmental effects. Seeds were collected from 4-8 maternal plants of each genotype for use in the experiment.

Experimental Design -- We examined the effects of genetic diversity and density on colonization success by factorially manipulating population genetic richness (1, 2, 4, or 8 genotypes) and plant density (low density vs. high density) in a common greenhouse environment. Low density populations consisted of eight individuals (0.05 indivs/cm^2), and high density populations had 16 individuals (0.10 indivs/cm^2). These densities are somewhat higher than those reported for adult populations of *A. thaliana* established in agricultural fields (0.001 - 0.02 indivs/cm^2, Goss 2005) but are lower than those observed in some invasive populations in North America (J. Stinchcombe, University of Toronto, *personal communication*). Each population was planted in a 12.5×12.5×6 cm (LxWxH) pot filled with Metromix 200 soil (Sun Gro Horticulture, Canada, Ltd.). Seeds were planted in a grid to ensure equal growing space. Each pot was placed within a larger soil-filled tray (30×30×6 cm) to simulate a founding population located in a disturbed, open habitat with no competitors. Plants could (and did) root through holes in the central pot to access soil in the larger tray.

A major component of plant colonization success is initial survival, or germination followed by seedling emergence. To accurately measure emergence, we carefully planted one *Arabidopsis* seed in each grid position by painting the seed onto the soil with a toothpick. In order to examine how genetic diversity affected population growth and reproduction independently of seedling emergence success, we also included a separate "overseeding" treatment in which, rather than planting a single seed, we sowed three to 10 or more seeds in each grid position using a pipettor and seeds suspended in water. After emergence, the extra plants in this "overseeding" treatment were weeded, leaving one plant per position and mimicking 100% seedling emergence.

We added additional replicates to allow partitioning of additive versus non-additive responses. Additive responses occur when there are no interactions among genotypes; in this case, population responses would be entirely predicted by summing the responses of their component genotypes in monoculture. Non-additive responses occur when there are interactions (e.g. facilitation, niche partitioning, competition) among genotypes that cause the population response to be significantly higher or lower than the sum of the responses of the component genotypes (Hughes *et al.* 2008). To allow partitioning of additive and non-additive effects of genetic diversity, all genotypes not randomly selected for the 1-genotype experimental treatment were grown in high and low density monocultures. The high density monocultures included both overseeding and no overseeding treatments. Therefore, monoculture populations of each genotype were replicated either three or four times. This resulted in 199 populations (4 genetic diversity levels x 2 density levels x 2 overseeding levels x 10 replicates, plus 13 monocultures x 2 density levels + an overseeding treatment for the 13 high density populations).

To minimize problems associated with nonindependence of replicates within a treatment, and increasing similarity among treatment levels as diversity increases (Huston & McBride 2002), genotypes were randomly chosen from a relatively large pool of 23 genotypes. Genotype combinations were then discarded (and new genotype combinations generated randomly) to meet the following criteria: replicates of the 1- and 2-genotype treatments were allowed no genotypes in common, replicates of the 4-genotype treatment were allowed only 1 genotype in common, and replicates of the 8-genotype treatment could share no more than 3 genotypes. EstimateS software (Colwell 2005) was used to calculate similarity indices. Similarity estimates were low, and compared favorably to those in other recent diversity experiments (Weltzin *et al.* 2003 and references therein): The average Jaccard coefficient of similarity within 4- and 8-genotype treatments was 0.06 and 0.178, respectively. Between treatments, the average Jaccard similarity coefficient was 0.075 for 2- and 4-genotype treatments, 0.084 for 2- and 8-genotype treatments, and 0.141 for 4- and 8-genotype treatments.

After planting, the populations were cold stratified at 4° C for eight days. Populations were then placed in the Rice University greenhouse on 28 November 2006. Temperatures in the greenhouse were allowed to vary with ambient temperatures (but were not allowed to fall below 10°C or exceed 29°C) to simulate a novel outdoor environment. Populations were watered as needed and no supplemental lighting or fertilization was implemented. The experiment was terminated when the majority (>80%) of the plants had senesced, on 4 April 2007.

Response Variables -- We assessed several estimates of population performance, including seedling emergence, biomass, flowering duration, and reproduction.

Population-level estimates of biomass and reproduction were calculated by summing values for the individual plants that comprised them. We scored seedling emergence percentage for each population in the no-overseeding treatment approximately four weeks following the end of the stratification period, after it appeared that most plants had germinated. Once the first plant bolted (21 December 2006), we recorded reproductive status (bolting, flowering, or producing fruits) of each plant in each population every two to three days until 25 January 2007. Then, we switched to recording reproductive status of all plants every seven days until early April. Flowering duration was calculated as the number of days between the initiation of flowering by the earliest flowering plant in a population and the initiation of flowering by the latest flowering plant in that population. In mid-April, all above-ground biomass (including rosettes, flowering stalks, seed pods, and any senesced leaves) was harvested, dried to constant weight, and weighed.

To estimate reproduction, allometric equations relating biomass to fruit production were developed for all 23 genotypes individually. At least seven plants from each genotype chosen randomly across treatments were assessed for fruit number and dry biomass; additional plants were then sampled until an $r^2 \geq 0.8$ was reached for each genotype (except genotype 8, for which $r^2 = 0.31$, $n = 44$ plants sampled). For statistical analyses involving genotype 8, actual fruit values for 44 plants were used, while allometric equations were employed for the 37 remaining individuals of that genotype.

Statistical Analyses – We tested for treatment effects on colonization success of the *Arabidopsis* populations using M/ANCOVA models that included the treatments genetic diversity (a continuous variable), density, overseeding, and all possible interactions (Proc GLM, SAS Institute 2003). Results treating genetic diversity as a fixed

categorical factor using M/ANOVA models did not differ from those obtained with genetic diversity as a continuous variable using M/ANCOVA models. The latter is standard practice for analyses of diversity (Tilman et al. 1996, Hughes & Stachowicz 2004, Reusch et al. 2005, Crutsinger et al. 2006, Crawford et al. 2007) and we have opted to retain this approach. In all models, the overseeding treatment never significantly affected the response variables (because seedling emergence was high and because plants with fewer neighbors were able to grow larger). Therefore, for clarity, this treatment was removed from the models and the final data analysis was limited to the effects of genetic diversity, density, and the diversity × density interaction.

The following response variables were examined: percentage seedling emergence (only for the populations with no overseeding), above-ground biomass, flowering duration, and fruit number. Following a MANCOVA finding significant treatment effects on all response variables considered together, we performed protected ANCOVA (Scheiner 2001) on each response. All data met assumptions of normality of residuals and homogeneity of variances, except for the analysis of fruit number, where two outliers were excluded to improve normality. To test if population performance for the four traits was positively correlated, the six pairwise correlations were examined for all populations in all treatments (except for percentage seedling emergence, where the overseeding treatment populations were excluded).

We then tested whether responses to genetic diversity were additive or non-additive in nature by conducting Monte Carlo simulations. Artificial populations matching the genotypic composition of each of the experimental polycultures were constructed by randomly sampling trait values (with replacement) from individual plants

growing in monoculture, following the general logic of Johnson *et al.* (2006) and Crawford *et al.* (2007). Sampling only occurred within a density level (e.g., a given artificial low-density population was constructed only from individuals in low-density monocultures). We then examined the distribution of trait values for 9,999 sets of artificial populations and calculated 95% confidence intervals. When actual means fell outside these intervals we inferred non-additive effects of genetic diversity. Monte Carlo simulations were programmed using SAS macro language (SAS Institute 2003); the code is available on request from the authors.

1.3 RESULTS

Increased genetic diversity within founding populations of *Arabidopsis* significantly increased population-level seedling emergence, biomass, flowering duration, and reproduction (Tables 1.2 and 1.3). In monoculture, 66% of the planted seeds emerged, compared to 82% of the seeds in the highest diversity treatment ($F_{1,75}$ = 12.24, P = 0.0008) (Figure 1.1A). This pattern arose because most genotypes showed increased per-capita germination rates in higher-diversity environments: 18 of the 23 genotypes (78%) responded positively to increased genetic diversity (i.e., showed significantly positive correlations between genetic diversity level and germination percentage). Populations with the highest genetic diversity also produced 69% more biomass than monocultures ($F_{1,155}$= 23.53, $P < 0.0001$) (Figure 1.1B). On average, the 8-genotype treatment flowered for 25 days longer than the 1-genotype treatment ($F_{1,155}$ = 43.46, $P <$ 0.0001) (Figure 1.1C), and produced ≈1400 (20%) more fruits ($F_{1,153}$ = 5.80, P = 0.0274) (Figure 1.1D).

Populations with high performance for one response variable did not necessarily perform well across all response variables (Table 1.4). Three of the five relationships between response variables were significantly positively correlated. Percentage seedling emergence was significantly correlated with both biomass (Pearson's $r = 0.23$, $P = 0.003$) and flowering duration ($r = 0.27$, $P = 0.0005$), and flowering duration was significantly correlated with biomass ($r = 0.25$, $P = 0.0014$). However, these correlations were generally weak. The correlation between biomass and fruit production is not presented, since fruit number was calculated from allometric equations using biomass as the predictor variable.

In contrast to the substantial effects of genetic diversity, plant density did not significantly affect percentage seedling emergence, biomass, or fruit production. This potentially counterintuitive result was the result of larger per-capita values for biomass and fruit production in low-density populations that compensated for lower absolute numbers of plants (data not shown). There was a marginally significant trend for longer flowering periods in high-density populations relative to low-density populations ($F_{1,155} = 3.45$, $P = 0.065$). There were no significant density by genetic diversity interactions (Tables 1.2 and 1.3), suggesting that the effects of genetic diversity did not depend on the initial population size or level of intraspecific competition tested in this experiment.

Significant non-additive effects of genetic diversity were detected for all four response variables. Diversity treatments containing either 4 or 8 genotypes of *Arabidopsis* emerged more often than expected under additivity (Fig 1.2). However, when populations contained only 2 genotypes, they germinated less often than expected under the additive model. All diversity levels produced significantly more biomass than

predicted from the additive model, with the most diverse (8-genotype) treatment massing 30% more than expected under non-additivity (Fig 1.2). Populations showed significant positive non-additive responses for flowering duration in the 2- and 4-genotype (but not 8-genotype) treatments (Fig 1.2). Diversity treatments containing either 2 or 8 genotypes produced more fruits than expected, and the highest diversity treatment produced nearly 1300 more fruits than expected under additivity (Fig 1.2).

1.4 DISCUSSION

Our results show that higher levels of genetic diversity within experimental founder populations of *Arabidopsis thaliana* are associated with increased initial seedling emergence, flowering duration, biomass, and reproduction. The lack of strong correlations between the response variables indicates that positive effects of genetic diversity on population performance accrued during multiple stages of the plants' life cycle. The patterns are influenced by an interaction among the genotypes in a population, as evidenced by the non-additive effects of diversity on all responses. In contrast, density had no significant effect on any of the measured responses, nor did it modify the effect of genetic diversity on these responses. This suggests that the interactions occurring among genotypes that produced non-additive responses were present at both density levels. Our results suggest that, on a short-term, "ecological" timescale, high levels of genetic diversity could aid a population colonizing a new habitat by increasing the probability the population will survive, grow, and reproduce under novel conditions. Thus, the ecological consequences of genetic diversity and admixture in founder populations may be profound and determine whether the longer-term effects of genetic diversity on adaptation ever come into play.

Consequences of genetic diversity -- While several recent ecological studies have examined the relationship between genetic diversity and population processes (Newman & Pilson 1997, Hughes & Stachowicz 2004, Gamfeldt et al. 2005, Reusch *et al.* 2005, Johnson *et al.* 2006, Crutsinger *et al.* 2006), these studies have not specifically addressed how genetic diversity within populations influences colonization success. They have employed genotypes of the focal species that evolved in a common environment within the species' native range and do not always measure the population variables relevant to colonization success. Populations of colonizing species may frequently be composed of distantly related genotypes from multiple locations in the range of the species, and furthermore are likely to face novel biotic and abiotic conditions. Under these conditions, we found that genetic diversity is capable of influencing colonization success.

While a positive effect of increased genetic diversity on population biomass production has previously been documented (e.g. Crutsinger *et al.* 2006), to our knowledge, no study has found that genetically diverse populations display significantly higher reproduction. Johnson *et al.* (2006) found that some genotypes of *Oenothera biennis* had a greater fitness when grown in diverse populations relative to monocultures. We found that this response scaled up to the population-level, with more diverse populations producing more fruits than less diverse populations.

We also found that higher levels of genetic diversity promoted flowering duration, a novel result. This characteristic is likely to be an important determinant of colonization success for many plant species; a longer flowering period increases the chances that a population will overlap with pollinators that may vary in seasonal abundance (Rathcke & Lacey 1985). This could be critically important in novel environments, as co-evolved

pollinators are unlikely to be present; however, pollinator attraction is clearly less important for highly self-compatible species such as *Arabidopsis thaliana* (Abbott & Gomes 1989).

Our finding that genetic diversity influenced seedling emergence success is another novel and perhaps counterintuitive result. We hypothesize that seed-seed or seedling-seed interactions are responsible. There is a substantial literature documenting seed-seed and seedling-seed interactions (e.g. Bergelson & Perry 1989, Murray 1998, Inouye 1980, Dyer et al. 2000, Lortie & Turkington 2002, Turkington et al. 2005). In these experiments, the emergence behavior of seedbank seeds (either percentage or timing) is altered by their density or seedling density. For example, Murray (1998) manipulated seed density in *Eragrostis curvula* and found that seeds planted at higher densities emerged at higher per-capita rates. Further experiments using leachates are consistent with soilborne chemical cues; for example, Bergelson & Perry (1989) found that leachate from germinating seeds accelerated emergence timing of *Senecio vulgaris* and *Capsella bursa-pastoris* seeds, relative to plain water. In our system, we hypothesize that high-diversity treatments are more likely to contain one or more early-germinating genotypes, and that these early germinants modify the chemical environment of the remaining seedbank and cause an increase in emergence percentage. Currently we are conducting further experiments to test this hypothesis, to isolate the mechanism, and to examine whether such behavior may be adaptive.

Despite the growing body of experimental evidence that genetic diversity has important ecological consequences, few studies have examined the importance of genetic diversity relative to other population-level factors (Hughes *et al.* 2008). This is a critical

next step in judging the importance of genetic diversity for ecological processes. In our experiment, we manipulated the density of individual plants as well as genetic diversity. We hypothesized that these factors are important because they could alter the strength of interaction among individuals. We found no significant effect of density on any of the response variables, except for a trend for high density populations to flower longer than low density populations (Table 1.3). Therefore, we conclude that genetic diversity was a more important driver of colonization success than density for *A. thaliana* under our experimental conditions.

Additive and non-additive effects of genetic diversity -- We found that non-additive effects generally led to increased population performance in the founder populations. Non-additive effects were found in 10 of the 12 comparisons, with the exceptions being the 8-genotype treatment for flowering duration and the 4-genotype treatment for fruit production. Only with the 2-genotype treatment for seedling emergence percentage was a significant negative, non-additive effect detected. This pattern could have been caused by competition between early-germinating and late-germinating genotypes. Other work has found significant non-additive positive effects of genetic diversity for population biomass (Crutsinger *et al.* 2006), but we also document non-additive effects for population seedling emergence percentage, flowering duration, and fruit production. This suggests that positive interactions among genotypes may be critically important for population survival and growth in novel conditions. Positive interactions that may be occurring in this system include resource partitioning and facilitation. Resource partitioning can occur when genotypes utilize resources at different rates, leading to more efficient utilization of the available suite of resources. Since

resource partitioning assumes that competition for resources among individuals within a genotype is stronger than competition between genotypes, individuals in more diverse populations would suffer less from competition, allowing them to maximize growth and fitness. Facilitation may occur when the presence of one genotype modifies the environment in a way that benefits other genotypes. The presence of a beneficial genotype in a more diverse population could lead to greater individual growth and fitness. Further experimentation on this system could elucidate which of these factors contribute to the positive, non-additive responses we found.

Additive effects of diversity can also be inferred from the randomizations. For biomass, flowering duration, and fruit production, as the number of genotypes present in the populations increases, so does the expected mean of the response. For example, the mean of flowering duration makes an obvious shift from approximately 59 days when two genotypes are present to almost 71 days when eight genotypes are present (Figure 1.2). Additive effects of diversity could be attributed to the sampling effect, where individuals with a relatively large effect on the response are more likely to be included in more diverse populations. For example, populations with higher diversity have a greater likelihood of containing genotypes that flower very early and very late, effectively increasing the duration of the flowering period.

Caveats – Since our experiment was conducted in a very controlled environment, an interesting question is how the effects of genetic diversity will change in magnitude and direction under more complex ecological scenarios, such as in field situations. For example, mixtures of distinct plant genotypes are known to alter disease dynamics in crop plants (Mundt 2002). Similarly, when grown in polyculture, herbivore-

susceptible genotypes of a plant may benefit from associational resistance when growing next to less susceptible genotypes (*sensu* Tahvanainen & Root 1972). Alternatively, particularly attractive genotypes may negatively affect more resistant plants via associational susceptibility, as has been found for attack of a galling midge (*Rhopalomyia solidaginis*) on genotypes of *Solidago altissima* (Crawford *et al.* 2007). Thus, genetic diversity will likely have complex effects on colonization success in populations subject to disease, pest attack, and other abiotic and biotic factors.

A second caveat arises from the choice of genotypes used in the experiment. Given that genotypes were drawn from a wide geographic range and have known phenotypic differences (e.g., in size and flowering time; ABRC, Columbus, Ohio), effects of genetic diversity found in this study could be larger than that associated with typical founder populations. However, one can also imagine founder populations in which very high levels of phenotypic diversity would be present, for example, introductions of ornamental plants in which morphological diversity is explicitly sought (see below). For *A. thaliana* in particular, a recent analysis of the genetic structure of Eurasian populations suggests that they are isolated by distance (Beck *et al.* 2008). Therefore, if multiple introductions from several source populations occurred, relatively high levels of genetic diversity could result.

Conservation implications – Our finding that increased genetic diversity leads to increased colonization success in our experimental system suggests that admixed founder populations of exotic species may have improved ability to become established. Several studies of successful invasive species have found that populations are characterized by a relatively large amount of genetic diversity (e.g., Kolbe *et al.* 2004, Genton *et al.* 2005).

High levels of genetic diversity are likely found in species that have been introduced multiple times to an area. Such admixture may be exceedingly common during particular types of dispersal events; for example, Roman and Darling's (2007) review found that 66% of reports on invasions mediated by ballast water showed levels of within-population genetic diversity at least as high in the introduced range as the native range. Similarly, agriculturally or horticulturally important species may become invasive after introductions of distinct genotypes with different desirable qualities. For example, many genotypes of reed canary grass (*Phalaris arundinacea*) were introduced to Eurasia and North America for forage and soil stabilization (Lavergne & Molofsky 2004).

In general, our results suggest that population–level characteristics should be considered in addition to the individual-level traits (e.g., growth rate, dispersal ability, and generation time; see Whitney & Gabler 2008 for a review) that are the typical focus of invasive species risk assessment schemes. Furthermore, exclusion, quarantine and control procedures for invasive species would likely benefit from practices that limit admixture or focus on species prone to admixture.

Conclusion -- While much theory suggests that genetic diversity should allow populations of colonizing species to adapt to their new environments (Sakai *et al.* 2001, Lee 2002, Holt *et al.* 2005, Novak & Mack 2005, Dlugosch & Parker 2008), little thought has been given to the short-term ("ecological") consequences of genetic diversity that precede any evolutionary changes. Both additive and non-additive effects were important determinants of increased colonization success in our system, suggesting that both genetic identity of the colonists and interactions among genotypes may have profound influences on the relative success or failure of a colonization event. The genetic diversity

present in colonizing populations may be a useful metric for predicting colonization success.

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1.6 CITED LITERATURE

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1.7 TABLES

Table 1.1 Arabidopsis accessions included in the experiment.

Number	ABRC Stock Number	Name	Country
1	CS6643	Burren	Ireland
2	CS6660	Canary Islands	Spain
3	CS1084	Coimbra	Portugal
4	CS6673	Columbia	USA
5	CS6674	Catania	Italy
6	CS22614	Cape Verdi Islands	Cape Verdi
7	CS6688	Edinburgh	United Kingdom
8	CS1144	Espoo	Finland
9	CS6736	Hilversum	Netherlands
10	CS20	Landsberg erecta	Germany
11	CS6792	Mühlen	Poland
12	CS1380	Martuba	Libya
13	CS6805	Nossen	Germany
14	CS22661	New Zealand	New Zealand
15	CS6824	Oystese	Norway
16	CS6839	Poppelsdorf	Germany
17	CS6850	Rschew	Russia
18	CS6857	San Feliu	Spain
19	CS6874	Tsu	Japan
20	CS6889	Wilna	Russia
21	CS6891	Wassilewskija	Russia
22	CS6897	Wü	Germany
23	CS690	Zurich	Switzerland

Table 1.2 MANCOVA results for the effects of genetic diversity and density on *Arabidopsis* population-level seedling emergence, biomass, and reproduction. Bold P-values are significant at $P < 0.05$.

	d.f.	Pillai's Trace	*F*	*P*
Genetic Diversity	1, 150	0.2890	15.24	**<0.0001**
Density	1, 150	0.0274	1.06	0.3783
GD X Density	1, 150	0.0222	0.85	0.4945

Table 1.3 ANCOVA results for the effects of genetic diversity and density on *Arabidopsis* population-level seedling emergence, biomass, and reproduction. Bold P-values are significant at $P < 0.05$.

	% Seedling Emergence				Aboveground Biomass				Flowering Duration				Number of Fruits			
	d.f.	***F***	***P***	R^2	***d.f.***	***F***	***P***	R^2	***d.f.***	***F***	***P***	R^2	***d.f.***	***F***	***P***	R^2
Model	3,75	4.77	**0.0042**	0.16	3,155	9.11	**<0.0001**	0.15	3,155	17.05	**<0.0001**	0.25	3,153	3.05	**0.0303**	0.06
Genetic Diversity	1	12.24	**0.0008**		1	23.53	**<0.0001**		1	43.46	**<0.0001**		1	4.96	**0.0274**	
Density	1	0.52	0.4724		1	0.34	0.5585		1	3.45	0.065		1	0.00	0.9460	
GD X Density	1	1.71	0.1944		1	0.36	0.5521		1	0.11	0.7354		1	1.28	0.2606	

Table 1.4 Pearson correlation coefficients between response variables for all populations in all treatments, except for percentage seedling emergence (where overseeding treatment populations were excluded) . Correlation coefficients are above the diagonal and P-values are below. Bold P-values are significant at $P < 0.05$.

	Percentage Seedling Emergence	Biomass	Flowering Duration	Fruit Production
Percentage Seedling Emergence		0.23	0.27	0.08
Biomass	**0.0030**		0.25	0.65
Flowering Duration	**0.0005**	**0.0014**		0.11
Fruit Production	0.325	**<0.0001**	0.1429	

1.8 FIGURES

Figure 1.1 Response of population-level seedling emergence, biomass, and reproduction to genetic diversity. Open circles are population-level values. In panel A, an individual circle represents on average 2.2 populations (range 1-8); circles overlap completely because seedling emergence was necessarily scored in discrete intervals. Black squares are treatment means (± SE) and are offset for clarity. Standard errors are associated with the least-square means from a model containing all treatments (diversity, density, overseeding).

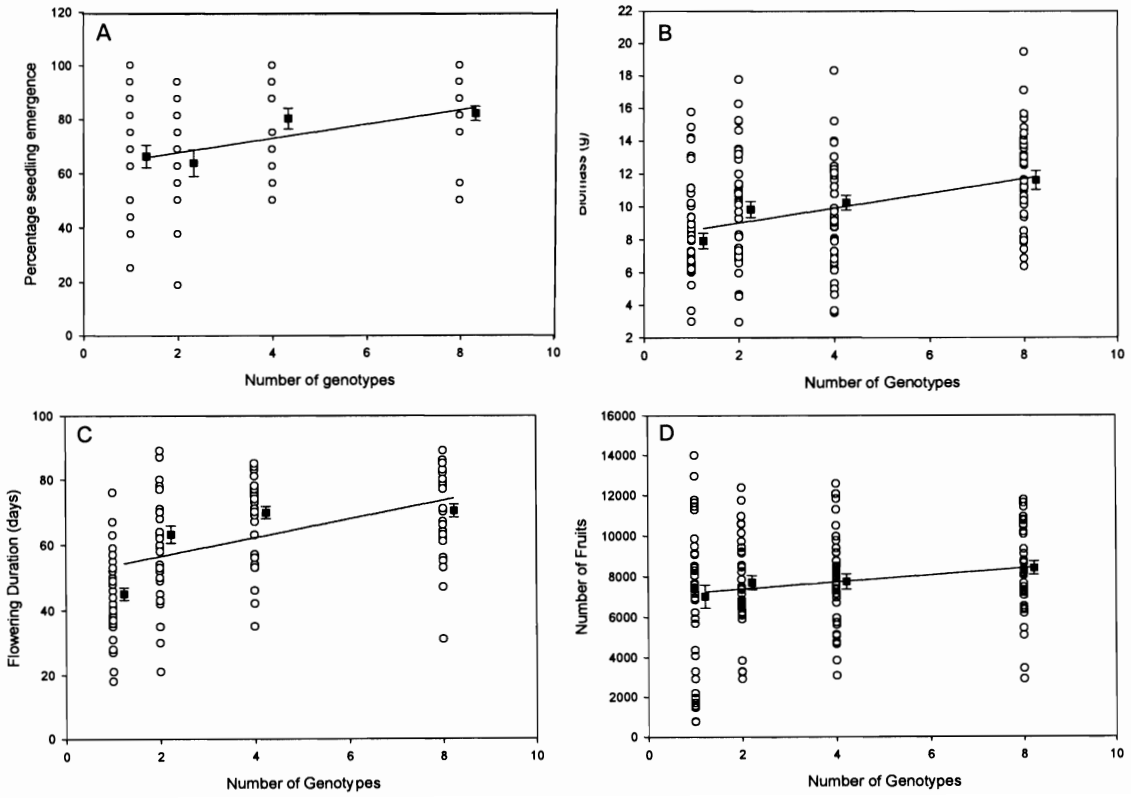
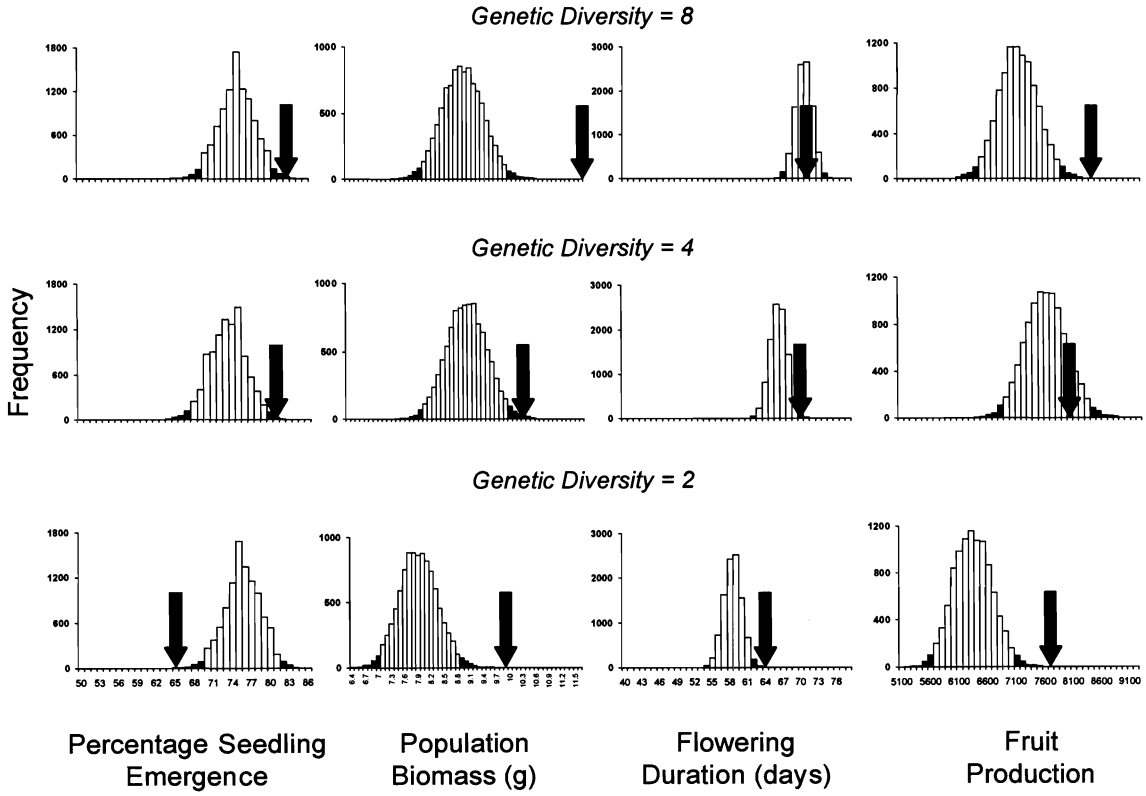


Figure 1.2 Non-additive effects of genetic diversity for four population-level response variables: percentage seedling emergence, flowering duration, biomass, and number of fruits. Shown are the distributions of trait values for 9,999 sets of artificial populations, constructed in Monte Carlo simulations by randomly sampling response variable values from individual plants growing in monoculture. Shaded bars highlight the area outside of the 95% confidence intervals. Arrows indicate the location of the actual (observed) means.



Chapter 2

Plant Species Diversity and Genetic Diversity Interactively Affect Primary Productivity

Biodiversity plays an important role in ecosystem functioning. Both plant species diversity and genetic diversity can affect community properties and ecosystem processes. However, the relative contribution of species diversity and genetic diversity to ecosystem functioning is relatively unknown and interactions between the two levels of diversity have not been detected. Interactive effects between genetic diversity and species diversity are particularly important to investigate, because if interactions between the two levels of diversity exist, the magnitude of the effect of biodiversity loss could be greater than previously estimated. Here, we addressed how plant species diversity and genetic diversity independently and interactively influenced a key ecosystem function, primary productivity, utilizing multi-year common garden experiments. Our results show, for the first time, that aboveground biomass production can be mediated by an interactive effect between species diversity and genetic diversity in a dominant plant species, *Ammophila breviligulata*. Specifically, aboveground biomass increased with *A. breviligulata* genetic diversity, but only when more than one species other than *A. breviligulata* was present. When the dominant species was present, there was also a significant main effect of species diversity, which was negatively correlated with aboveground biomass. These patterns were influenced by net negative interactions among individuals in some treatment combinations, as evidenced by significant negative non-additive effects of diversity. In contrast, when plant species diversity and genetic diversity were independently manipulated, there was no significant effect of either level of diversity on

biomass production. Our results suggest that interactions between species diversity and genetic diversity are key to understanding the controls on productivity in this system, highlighting the importance of incorporating variation within species into our understanding of how communities are structured and ecosystems function.

2.1 INTRODUCTION

Currently, native diversity is being lost at a rapid rate due to many anthropogenic causes, including habitat destruction, pollution, and the spread of non-native species (Pimm et al. 1995, Chapin et al. 2000). This loss has spurred research over the last two decades to elucidate the influence of biodiversity on ecosystem functioning (reviewed by: Loreau et al. 2001, Hooper et al. 2005). The majority of these studies have focused on how reductions in the number of plant species influence terrestrial ecosystems (Duffy et al. 2007) and have found that ecosystem functioning is generally negatively impacted as species richness is reduced (meta-analyses: Balvanera et al. 2006, Cardinale et al. 2006). However, before a species is lost from the community, it is likely that it will suffer losses in genetic diversity due to shrinking population sizes (Ellstrand and Elam 1993). In addition to having long-term effects on evolutionary potential, the loss of intraspecific diversity can have negative consequences on a shorter timescale, if variation within species (genetic diversity) plays an analogous role to variation among species (species diversity) in the maintenance of ecosystem functioning (Hughes et al. 2008). Furthermore, if interactions between the two levels of diversity exist, the magnitude of the effect of diversity loss could be greater than previously estimated.

While the consequences of intraspecific variation have only recently received much attention in the ecological literature, genetic diversity's importance for ecosystem

functioning has been clearly documented (reviewed by Hughes et al. 2008). Higher levels of genetic diversity increased the population biomass of goldenrod (*Solidago altissima*) in the field (Crutsinger et al. 2006) and of *Arabidopsis thaliana* in the greenhouse (Crawford and Whitney 2010, Kotowska et al. 2010). Additionally, population genetic diversity was positively correlated with population resistance and resilience to disturbance in eelgrass (*Zostra marina*) (Hughes and Stachowicz 2004, Reusch et al. 2005).

Similar to mechanisms linking higher species diversity with enhanced ecosystem functioning, the mechanisms underlying the positive effect of genetic diversity on ecosystem function include additive and non-additive effects (Hughes et al. 2008). Additive effects occur when community responses can be entirely predicted by summing the responses of component individuals in monoculture. Therefore, populations or communities with greater diversity have an increased probability of including a genotype or species with a large effect on the measured response (lottery model or sampling effect). Non-additive effects, on the other hand, occur when interactions (e.g. facilitation, niche partitioning, competition) among community members cause individuals to perform differently in polyculture relative to monoculture. Positive non-additive effects can alter ecosystem functioning relative to the additive expectation and have been documented in experimental studies of genetic diversity. For example, diverse populations of *Arabidopsis thaliana* produced 30% more biomass than the additive expectation (Crawford and Whitney 2010), and positive non-additive effects of diversity increased goldenrod biomass above what was expected under an additive model (Crutsinger et al. 2006). Negative non-additive effects can also occur, for example, if

competition between more distant relatives is stronger than competition among close relatives.

Because of parallels in both the direction and magnitude of ecosystem responses to species diversity and genetic diversity, it has been suggested that the effect of genetic diversity may be as strong, if not stronger, than the effect of species diversity on ecosystem functioning (Crutsinger et al. 2006, Hughes et al. 2008). However, only one study has tested for relative effects by independently manipulating both levels of diversity in the same experiment (Cook-Patton et al. *in press*); here, the magnitude of the effect of genetic diversity in evening primrose (*Oenothera biennis*) on population-level biomass was comparable to the magnitude of the effect of species diversity on community-level biomass. Furthermore, only one study to date has manipulated both levels of diversity in combination. In a factorial experiment utilizing eight genotypes of eight plant species representative of limestone grasslands, Fridley & Grime (2010) found that plant genetic diversity did not alter the effect of species diversity on primary productivity. Thus, little is known about the relative importance of species diversity versus genetic diversity for mediating biomass production, and even less is known about the potential for interactive effects.

Interactive effects between genetic diversity and species diversity are particularly important to investigate, because natural systems are usually composed of several genotypes of many different species. Intraspecific variation is likely to alter interactions among species (Bolnick 1993). For example, there is considerable evidence that genetic identity plays a role in competition among plant species (Turkington and Harper 1979, Taylor and Aarssen 1990, Lankau and Strauss 2008). Genetic identity can also influence

the aboveground and belowground communities associated with plants (Bangert et al. 2006, Mooney and Agrawal 2008, Schweitzer et al. 2011), as can plant genetic diversity (Crutsinger et al. 2006, Johnson et al. 2006). These genetically-mediated changes in interaction strength and community composition may generate an interactive effect of species diversity and genetic diversity on ecosystem functioning. For example, the combination of high genetic diversity and high species diversity could alter ecosystem functioning more than the additive effects of either factor alone. Understanding the potential for interactive effects can improve predictions for the consequences of biodiversity loss and better inform restoration efforts.

Here, we addressed how plant species diversity and genetic diversity (within a dominant species) independently and interactively influenced a key ecosystem function, primary productivity, utilizing multi-year common garden experiments. Specifically, we addressed the following questions: (1) Do interactions between plant species diversity and genetic diversity alter primary productivity? (2) What is the relative importance of each level of diversity in affecting primary productivity?

2.2 METHODS

Study system – This experiment was conducted in the dune system surrounding Lake Michigan at Sleeping Bear Dunes National Lakeshore (44° 43.689' N, 86 ° 07.369' W). Great Lakes sand dunes support plant communities of relatively low species richness (1-5 species/m^2, Crawford, unpublished data, Cowles 1899), making this an ideal ecosystem for realistic, yet feasible manipulations of species diversity. Dune species comprise a variety of functional types, including several grasses, woody species, and forbs. The dominant plant species, *Ammophila breviligulata* (American beachgrass),

grows primarily via ramets and acts as an ecosystem engineer by stabilizing sand during primary succession, which then allows other plants to colonize (Olson 1958, Cheplick 2005). Natural populations of *A. breviligulata* are typically composed of 1-5 genotypes per m^2 (Fant et al. 2008).

Plant material – We collected all plant material from Sleeping Bear Dunes National Lakeshore during July 2007. *Ammophila breviligulata* ramets were collected from 14 populations that were separated by at least 1km and grown at a commercial nursery that specializes in the propagation of *A. breviligulata* for ecological restoration (VansPines Nursery, Holland, Michigan, USA). Nine other plant species were collected for the manipulation of species diversity. These included four grasses (*Calomovilfa longifolia*, *Elymus canadensis*, *Koeleria pyramidata*, and *Schizachyrium scoparium*), four woody species (*Arctostaphylos uva-ursi*, *Prunus pumila*, *Vitis riparia*, and *Salix cordata*) and a forb (*Asclepias syriaca*). The woody species were propagated from cuttings collected from 3-5 mature individuals. For the other species, material was collected from a single population. All cuttings and seeds were propagated at a commercial nursery in Michigan (Richey Nursery Company, LLC, Spring Lake, MI, USA), with the exception of *C. longifolia* and *K. pyramidata,* which were collected near the common garden and directly transplanted into the plots.

Characterizing genetic diversity – To ensure the validity of our genetic diversity treatment, we examined the genetic diversity within and among the populations of *A. breviligulata* using intersimple sequence repeat (ISSR) markers. These highly variable nuclear markers have previously been used to describe populations of *A. breviligulata* (Fant et al. 2008), as well as other species (Wolfe et al. 1998a, 1998b, Esselman et al.

1999). We used the three primers employed by Fant et al. (2008) - (GA)8T, (GA)8C, and (CA)8G – to genotype 8 individuals each from our 14 populations of *A. breviligulata.* Resulting bands were scored as either present or absent and analyzed using ANOSIM (PRIMER v6, Clarke and Gorley 2006) which showed that genetic variation among populations was greater than genetic variation within populations (Global $R = 0.80$, $P < 0.01$). Additionally, pair-wise contrasts revealed that all populations except two (3 and 12) had significantly different banding patterns ($P < 0.05$, Appendix A, Table 1). These two populations never occurred together in treatments with three populations of *A. breviligulata* and only occurred together in 2 of 28 plots with six populations of *A. breviligulata.* These results confirmed that by increasing the number of populations in a community, we increased genetic diversity within *A. breviligulata.* Additional details of the molecular and statistical analysis can be found in Appendix A.

Common garden – The common garden was established at a site where the National Park Service demolished homes in 2004 to perform a restoration of the dune habitat. Few plants had colonized the area since demolition ($<0.25/m^2$); non-native species were manually removed and native species were relocated prior to plot establishment. Due to differences among species in optimal planting time, we established the experiment in three phases. We planted *Ammophila breviligulata* in mid-October 2007, because local land managers reported greater success with fall plantings. The following June, we planted the woody species, and we planted the remaining species in July. Plots were watered during the summer of 2008 to promote establishment, and were weeded monthly during the growing seasons to maintain diversity treatments.

Genetic diversity x species diversity manipulation (crossed plots) – To examine interactions between species diversity and genetic diversity, we factorially crossed three levels of species diversity (1, 3, or 6 species) with three levels of genetic diversity within *A. breviligulata* (1, 3, or 6 populations) (Figure 2.1). We established treatments in 1.5m X 1.5m plots at a density of 24 plants per plot, comprised of 12 individuals of *A. breviligulata* and 12 individuals of the other species, to create a realistic density and composition for this community. Therefore, the species diversity treatments describe the species richness of the plot, excluding *A. breviligulata.* Individuals in the plots were randomly assigned to a position in a staggered grid design to maximize the number of interactions among plants. Within the species diversity and genetic diversity treatments, we planted equal numbers of individuals for each species/population. For example, in a plot with a treatment combination of 3 species and 6 populations, we planted 4 individuals of each of the 3 species other than *A. breviligulata* and 2 individuals of each *A. breviligulata* population. Each treatment combination was replicated 7 times for a total of 63 plots.

To minimize the potential for quasi-replication - the replication of a specific community in the highest diversity treatment that confounds diversity effects with community composition effects (Huston & McBride 2002) - *A. breviligulata* populations were selected from a pool of 14 and other species were selected from a pool of nine. Populations and species were chosen randomly. However, to avoid increases in the similarity of communities at high diversity levels, random combinations were chosen to maximize dissimilarity within treatment combinations. For example, replicates containing 6 populations of *A. breviligulata* and 3 species were allowed to have 2 of the

14 populations in common and 2 of the 9 species in common. If a treatment replicate deviated from these stipulations, the replicate was discarded and a new replicate was randomly generated.

Independent diversity manipulations and monocultures (independent plots) – In our simultaneous manipulations of diversity, plots contained equal numbers of both the dominant and non-dominant species, whereas in our independent manipulations of diversity the dominant plant was either present or absent. We expected that diversity effects may differ between plots simultaneously manipulating diversity and plots independently manipulating diversity due to variation in the presence of the dominant plant. Thus, we established additional plots that manipulated only species diversity or genetic diversity (1, 3, or 6 species/populations) to measure their independent effects (Figure 2.1). Plots with 3 or 6 species/populations were established at the same size and density as the crossed diversity plots. Plots containing only one species or one population (monocultures) were established to obtain individual additive effects on biomass production and allow the partitioning of additive versus non-additive effects. Due to space and labor limitations, monocultures were planted at the same density as individuals in the diversity plots, but with 12 individuals per plot. Each population monoculture (14 total) and species monoculture (9 total) was replicated three times, and independent diversity plots were replicated seven times, for a total of 97 plots. As with the species diversity x genetic diversity plots, the potential for quasi-replication was minimized and dissimilarity within treatment combinations was maximized.

Biomass measurements – Aboveground biomass was estimated non-destructively using allometric equations developed for each species from destructive harvests (see

appendix B). This method makes the assumption that allometry is not affected by plant diversity, but this method has been used before for estimating biomass in diversity studies (Crutsinger et al. 2006). We used a minimum of 16 plants per species to construct the equations. All correlations were significant at $P < 0.0001$, and explained at least 88% of the variation in plant weight for all species except *A. syriaca* ($r^2 = 0.78$). Populations of *A. breviligulata* in the common garden varied significantly in maximum height and tiller number (height: $F_{13,49} = 2.16$, $P = 0.0265$; tiller number: $F_{13,49} = 3.93$, $P = 0.0002$), which produced variation in estimated aboveground biomass from the *A. breviligulata* allometric equation. After the experiment had been fully established for one year, we measured every plant in each plot once per month during the growing season (June, July, and August 2009). To calculate community-level biomass, values for individual plant biomass were summed for each plot.

Below-ground biomass was estimated during August 2010. Eight soil cores (33cm length x 2.22cm diameter) were taken at equal intervals along the diagonal of each plot. The cores were combined, and the samples were homogenized then transported to Rice University (Houston, TX, USA), where they were stored at 4° C for one month until belowground biomass was extracted. Using a 200mL subsample of soil dried at 60° C, the soil was dry sieved with a mesh size of 1mm to remove roots. Then, roots were separated from soil aggregates and weighed to obtain mg of roots per 200mL soil. This procedure was repeated for one additional 200mL subsample per plot, and the two values were averaged before data analysis.

Statistical analyses: Diversity effects – In the crossed plots (plots simultaneously manipulating species diversity and genetic diversity), we tested for the effects of diversity

and time on total estimated aboveground biomass using a repeated measures mixed model. The model included the continuous effects of species diversity and genetic diversity, the fixed effect of time, and all possible interactions (Proc MIXED, SAS Institute 2009). Results were tested over the plot-level error. We used the heterogenous Toeplitz structure to model the covariances. This model was chosen based on AIC. Standard errors and F-statistics were KR corrected (Kenward and Roger 1997). For belowground biomass, we used a general linear model with the continuous effects of species diversity and genetic diversity and the genetic diversity x species diversity interaction (ProcGLM, SAS Institute 2009).

In the independent diversity manipulation (plots containing only species diversity or genetic diversity manipulations) the monocultures contained only half the number of plants contained in diversity plots. Therefore, to compare across treatment levels, the community-level aboveground biomass values were halved for the diversity plots. To test how diversity influenced biomass production, we used the repeated measures mixed models described above, but with either the continuous effect of species diversity or genetic diversity included in the model (Proc MIXED, SAS Institute 2009). For belowground biomass, we employed the same general linear model described above, but included only species diversity or genetic diversity in the model. Additionally, we tested for a correlation between aboveground biomass and belowground biomass with plot as the unit of replication (Proc REG, SAS Institute 2009).

During the course of the experiment, some mortality occurred. Models incorporating mortality as realized diversity did not differ qualitatively from models using the initially planted diversity, so for ease of interpretation the latter models are

presented. In all models, values for aboveground and belowground biomass were log-transformed prior to analysis to meet assumptions of normality of residuals and homogeneity of variances.

Statistical analyses: Identity effects – To test how the identity of each plant species or population influenced aboveground biomass production, we analyzed data from the monoculture plots using the repeated measures mixed models described above, but with the fixed factor of either species identity or genetic identity, time, and all possible interactions (Proc MIXED, SAS Institute 2009). Belowground biomass was analyzed with the general linear models described above, but with the fixed factor of either species identity or genetic identity. Values for aboveground and belowground biomass were log-transformed prior to analysis to meet assumptions of normality of residuals and homogeneity of variances.

Statistical analyses: Additive vs. non-additive effects – When a significant effect of diversity was detected, we tested whether the effect was driven by additive or non-additive diversity effects by conducting Monte Carlo simulations (Johnson *et al.* 2006, Crutsinger *et al.* 2006, Crawford & Whitney 2010). Artificial *in silico* populations matching the composition (species composition and genotypic composition) of each of the experimental diversity plots were constructed by randomly sampling biomass values (with replacement) from individual plants growing in monoculture. We then examined the distribution of biomass values for 9,999 sets of artificial populations and calculated 95% confidence intervals for each treatment mean. When actual means fell outside these intervals we inferred non-additive effects of diversity – that is, the effect of diversity on biomass production was not explained solely by the composition of the community.

Monte Carlo simulations were programmed using SAS macro language (SAS Institute 2003) following methods in Crawford & Whitney (2010).

2.3 RESULTS

Aboveground Biomass

Interactive effects of diversity – In the crossed plots, species diversity interacted with genetic diversity to mediate aboveground biomass production (Table 2.1). Specifically, in the presence of three or six other dune species, aboveground biomass increased with increasing genetic diversity in *A. breviligulata* (Figure 2.2). When either three or six species were present, biomass was over 30% greater when six populations of *A. breviligulata* were present relative to one population. However, at the lowest level of species diversity, when only one additional species was present with *A. breviligulata*, aboveground biomass declined with increasing genetic diversity. Biomass was 27% lower when six populations were present relative to one population (Figure 2.2). These effects were consistent across the growing season, as indicated by the lack of significant interactions between plant diversity and time (Table 2.1). The species diversity x genetic diversity effect was largely driven by negative non-additive effects of diversity, indicating that individuals interact to influence biomass production (Figure 2.3). In plots with three or six dune species, the increase in biomass with higher genetic diversity was explained by a negative non-additive effect that occurred when only one population of *A. breviligulata* was present (Figure 2.3). Similarly, in plots with one additional dune species, the decline in biomass with greater genetic diversity was explained by a negative non-additive effect that occurred when 6 populations of *A. breviligulata* were present (Figure 2.3).

There was a significant main effect of species diversity on aboveground biomass production in the crossed plots (Table 2.1). On average, increasing species diversity decreased aboveground biomass production when *A. breviligulata* was present. Three species plots produced the least amount of biomass, 16% less biomass than plots with one species and 10% less biomass that plots with six species (Figure 2.4). Both additive and non-additive effects of species diversity were responsible for this decline. Based purely on the additive expectation, biomass tended to decline with increasing species diversity (Figure 2.4). This decline was magnified by a significantly negative non-additive effect of diversity on biomass production when three species were present (Figure 2.4).

Independent effects of diversity – In the independent plots, neither increasing species diversity nor increasing genetic diversity had a significant effect on aboveground biomass production (Table 2.1), suggesting that the effects of species diversity are stronger in the presence of the dominant species, *A. breviligulata*. The average aboveground biomass for communities in which only *A. breviligulata* genetic diversity was manipulated was 30.70±16.17g/m^2, and the average biomass for species diversity plots was 8.82±4.29g/m^2. The biomass of *A. breviligulata* was approximately 7% lower in July than in June or August, as indicated by a significant effect of time for the genetic diversity plots (Table 2.1).

Identity effect – There was no significant effect of *A. breviligulata* genetic identity on aboveground biomass (Table 2.1), but *A. breviligulata* was the most productive species in monoculture, producing, on average, approximately 50% more biomass than the next most productive species, *K. pyramidata* (Figure 2.5). In species monoculture plots, the identity of the species had a significant effect on aboveground biomass

production (Table 2.1). On average, *K. pyramidata* produced over 7 times more biomass than the least productive species, *S. scoparium* (Figure 2.5). *A. breviligulata* genetic monoculture biomass peaked in July (32.37±2.66g/m^2), as indicated by a significant effect of time (Table 2.1). For monocultures of the other species, July was peak biomass production for all species except *E. canadensis*, which produced the greatest amount of biomass during flowering in August (18.14±2.17g), as supported by a significant time × species identity interaction (Table 2.1).

Belowground Biomass

Across all plots, belowground biomass was significantly positively correlated with aboveground biomass, although there was substantial variation as indicated by the relatively low correlation coefficient (Appendix, Figure 2.C1; $F_{1,158} = 51.26$, $P < 0.0001$, $r = 0.49$). The correlation remained significant when two outliers were removed ($F_{1,156} = 37.34$, $P < 0.0001$, $r = 0.44$). However, neither plant diversity nor identity influenced the amount of belowground biomass (all $P > 0.12$, Appendix, Table 2.C2). On average, 40.81±2.47mg of belowground biomass was estimated per 200mL soil sample.

2.4 DISCUSSION

Our results show, for the first time, that primary productivity can be driven by an interactive effect between species diversity and genetic diversity in a dominant plant species. Specifically, aboveground plant community biomass increased with the genetic diversity of *A. breviligulata*, but only when more than one additional plant species was present. In our factorial manipulation of species and genetic diversity, there was also a significant main effect of species diversity, which reduced aboveground biomass in the presence of *A. breviligulata*. These patterns were influenced by net negative interactions

among individuals in the communities, as evidenced by the significant negative non-additive effects of diversity. In contrast, when plant species diversity and genetic diversity were independently manipulated, there was no significant effect of either level of diversity on biomass production. In the independent manipulations, the species in the species diversity treatment were grown in the absence of *A. breviligulata*, so this result supports the hypothesis that effects of plant species diversity are stronger in the presence of the dominant plant species in this system. While there were no conclusive results for the effect of diversity on belowground biomass, belowground biomass was generally positively correlated with biomass aboveground. Our results suggest that independently, species diversity and genetic diversity have little effect on plant biomass production, but that interactions between the two levels of diversity are key to understanding the controls on productivity in this system.

While several studies have examined the relationship between primary productivity and either plant species diversity (Tilman et al. 1996, Tilman et al. 2001) or genetic diversity (Crutsinger et al. 2006), ours is among the first to simultaneously investigate the independent and interactive effects of diversity on above- and belowground biomass production. Unlike Cook-Patton et al. (*in press*), who found that the magnitude of biomass increase was the same for plots manipulating either species diversity or genetic diversity within one target species, we found no significant effect of either species diversity or genetic diversity in our independent diversity manipulations. However, in contrast to the findings of Fridley and Grime (2010), we did find that species diversity and genetic diversity interactively influenced biomass production. Fridley and Grime (2010) manipulated genetic diversity within all species in their experimental

communities, whereas we only manipulated genetic diversity within the dominant plant species, *A. breviligulata*. Effects of genetic diversity on ecosystem function are predicted to be particularly important in foundation species, which tend to form monospecific stands where genetic diversity may be more analogous to species diversity (Whitham et al. 2006, Hughes et al. 2008) – although there is evidence that genetic diversity in non-dominant species can influence ecological responses (Johnson et al. 2006, Cook-Patton et al. *in press*). In our factorial experiment, *A. breviligulata* was planted as 50% of the total individuals in the plots, but by the time these measurements were recorded, it had produced over 80% of the total aboveground biomass. Therefore, by focusing our manipulation of genetic diversity on *A. breviligulata*, we were probably more likely to detect an interactive effect than had the manipulation also included several non-dominant species.

We found that negative non-additive effects of diversity contributed to both the significant interactive effect of species diversity and genetic diversity and the significant main effect of species diversity in crossed plots on aboveground biomass production. Negative non-additive interactions indicate that individuals were performing worse in polyculture than they were in monoculture. This could be a sign that exploitative competition for resources was more intense in these treatments, lowering average plant biomass, or could indicate interference competition in the form of allelopathy. Alternatively, indirect interactions could cause decreased biomass, including negative plant soil feedback (Bever et al. 1997) or increased herbivory or foliar pathogen loads, which can vary with plant diversity (Mitchell et al. 2002, Scherber et al. 2006, Stein et al. 2010).

It is also possible that negative non-additive effects are a result of temporal or abiotic effects. In experiments manipulating species diversity, complementarity, which occurs when species grown in polyculture outperform their monoculture averages, tends to increase through time (Cardinale et al. 2007). Since plants in the dune system are long-lived and productivity is low relative to the other systems where diversity-productivity relationships have been documented (e.g. old-field systems, Tilman et al. 1996, Tilman et al. 2001, Crutsinger et al. 2006, Johnson et al. 2006), we may expect to see non-additive effects shift to become more positive through time. Also, previous work suggests that plant mixtures may show negative non-additive effects under conditions of low fertility, but positive non-additive effects under high fertility conditions (Fridley 2002). Sand dunes are relatively harsh environments with low nutrients and soil moisture, especially recently colonized dunes (Lichter 1998, Crawford, *unpublished data*). Soil samples from plots prior to planting revealed no detectable nitrogen, (Crawford, *unpublished data*), and nitrogen levels did not reach 1mg/ha until 500 years into a dune chronosequence (Lichter 1998). Therefore, the observed negative non-additive responses may be due to the low productivity environment, and we may expect to see negative interactions decrease through time as nutrients and soil moisture increase. Conducting similar experiments across a range of productivities could help elucidate the conditions under which negative versus positive non-additive effects of diversity prevail.

While positive non-additive effects of diversity are more often found to contribute to diversity-productivity relationships (Crutsinger et al. 2006, Cardinale et al. 2007), negative non-additive effects have been documented. In a disturbed grassland community, three annual plant species produced less biomass when grown in mixture

than when grown in monoculture (Polley et al. 2003). Similarly, negative interactions between algal species caused them to produce less biomass when grown together than when grown separately (Zhang and Zhang 2007). Negative non-additive effects of diversity have also been documented for other ecosystem responses. For example, mass loss during decomposition can be slower for species mixtures than species monocultures (McArthur et al. 1994, Nilsson et al. 1999).

Despite finding evidence that diversity influenced aboveground biomass production, we found no effect of plant diversity on belowground biomass. Previous studies manipulating plant species diversity have found conflicting results, with belowground biomass increasing with species richness in some cases (Reich et al. 2001, Tilman et al. 2001, Craine et al. 2003), and not responding in others (Hooper 1998, Wardle et al. 1999, Spehn et al 2000, Gastine et al. 2003, Bessler et al. 2009). The lack of response we observed could be explained by alterations in root:shoot in different diversity treatments that we could not detect (see Bessler et al. 2009 and references therein). Alternatively, belowground biomass is difficult to estimate, particularly for plants that produce most of their biomass belowground and root deeply into the soil, such as sand dune plants. Because we found that aboveground biomass was correlated with belowground biomass, we are optimistic that our sampling provided a realistic estimate of belowground biomass. However, variation in measurement within a plot was high (plot measurements, on average, had a standard error that was 25% of the average), and the correlation with aboveground biomass was not tight. More detailed sampling, including root excavations and deeper cores could provide a better test of belowground effects, but are more destructive and logistically more challenging.

Conclusion – Several studies have shown that, independently, plant species diversity (Tilman et al. 1996, Tilman et al. 2001) and genetic diversity (Crutsinger et al. 2006, Hughes and Stachowicz 2009, Crawford and Whitney 2010) can influence primary productivity; however, few studies to date have investigated the relative importance of these two levels of diversity (Cook-Patton et al. *in* press) or their potential for interactive effects (Fridley and Grim 2010). Here, we showed for the first time that plant species diversity and genetic diversity within a dominant species interactively affect aboveground biomass production, via non-additive diversity effects. This result clearly has important implications for conservation, as it suggests that preservation of both levels of diversity may be important for the maintenance of ecosystem functioning. It also suggests that future ecological research investigating interactions between the two levels of diversity should be conducted in order to gain a more holistic understanding of how communities are structured and how ecosystems function.

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2.7 TABLES

Table 2.1 Results from mixed models testing how plant diversity influenced aboveground biomass production throughout the field season. "Crossed GD x SD" includes plots with both levels of diversity manipulated. "GD only" and "SD only" include plots where only genetic diversity or species diversity were manipulated. The effects of G ID and S ID were analyzed using only monoculture plots. Bold *P*-values are significant at $P < 0.05$.

Aboveground Biomass				
		df	***F***	***P***
Crossed GD x SD	**Genetic diversity**	1, 59	1.99	0.1634
	Species diversity	1, 59	4.31	**0.0422**
	GD x SD	1, 59	4.55	**0.0371**
	Month	2, 69	1.60	0.2085
	Time x GD	2, 69	0.15	0.8628
	Time x SD	2, 69	0.58	0.5631
	Time x GD x SD	2, 69	0.27	0.7630
GD only	**Genetic diversity**	1, 54	0.07	0.7864
	Time	2, 70	7.26	**0.0014**
	Time x GD	2, 70	0.45	0.6373
SD only	**Species diversity**	1, 39	0.00	0.9712
	Time	1, 49	2.38	0.1032
	Time x SD	1, 49	0.37	0.6944
G ID	**Genetic identity**	13, 28	1.30	0.2721
	Time	2, 35	11.22	**0.0002**
	GID x Time	26, 39	0.89	0.6122
S ID	**Species identity**	8, 18	9.69	**<0.0001**
	Time	2, 23	57.51	**<0.0001**
	SID x Time	16, 25	2.95	**0.0077**

2.8 FIGURES

Figure 2.1 Diagram of the experimental design for the common garden. Circles with different patterns represent different populations of *Ammophila breviligulata*. Triangles with different patterns represent different plant species. In the independent plots, only one level of diversity, either genetic diversity within *A. breviligulata* or species diversity, was manipulated. In the crossed plots, both species diversity and genetic diversity within *A. breviligulata* were simultaneously manipulated. Plots were composed of ½ *A. breviligulata* and ½ other species. All plots contained 24 plants, except for monocultures, which had 12. Individuals within each plot were randomized and planted equidistant from one another in staggered rows to increase the number of interactions among individuals.

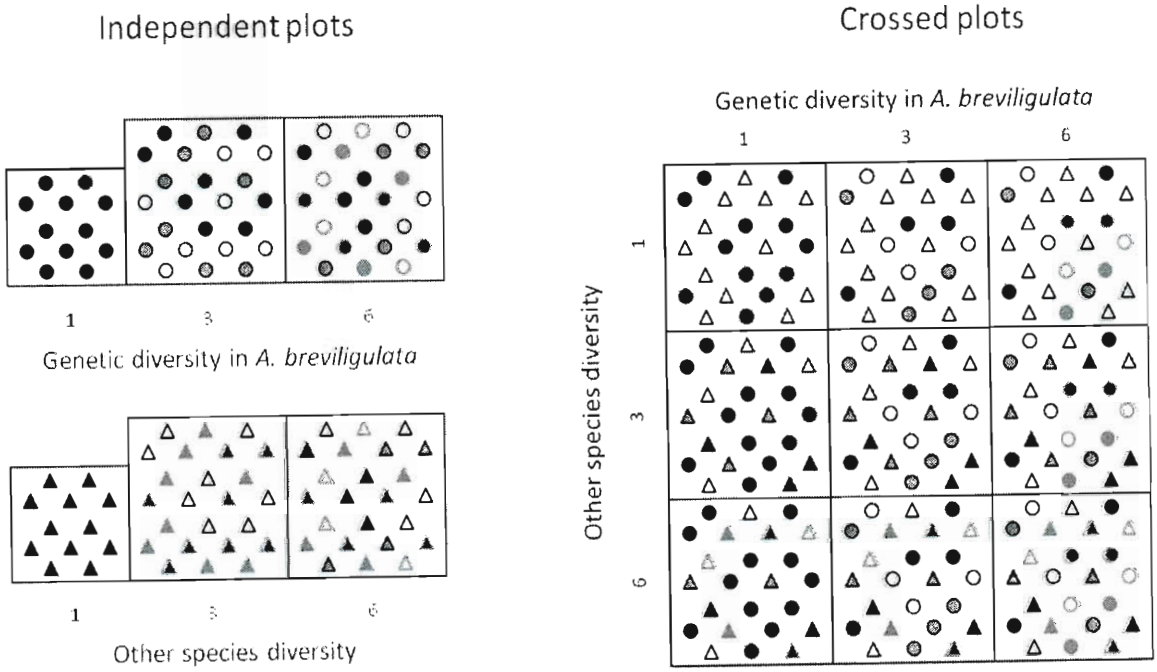


Figure 2.2 The average interactive effect of species diversity and genetic diversity within *A. breviligulata* on aboveground primary productivity in crossed plots.

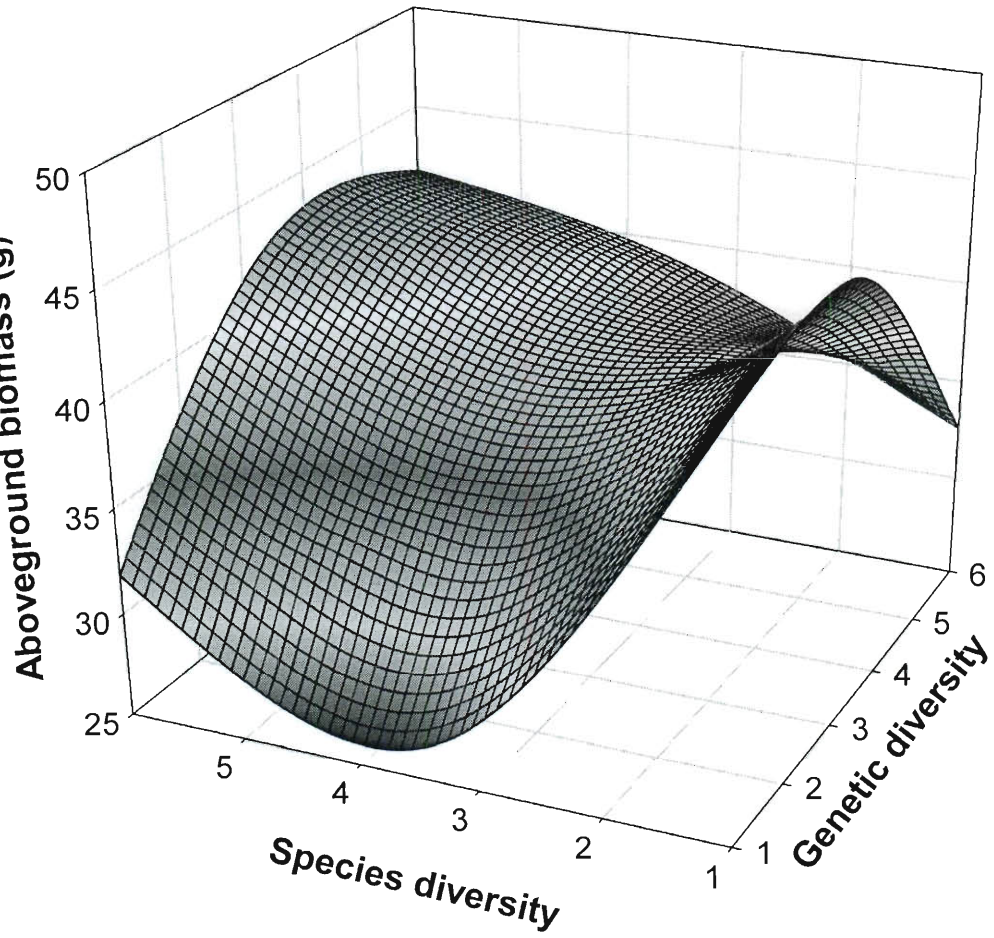


Figure 2.3 Additive expectations versus experimental treatment means for the significant interaction between species diversity and genetic diversity on aboveground biomass production. Triangles are the additive expectation based on 10,000 Monte Carlo simulations surrounded by 95% confidence intervals. Actual treatment means are represented by circles and are slightly offset for visual clarity. Asterisks indicate values that differ significantly from the additive expectation.

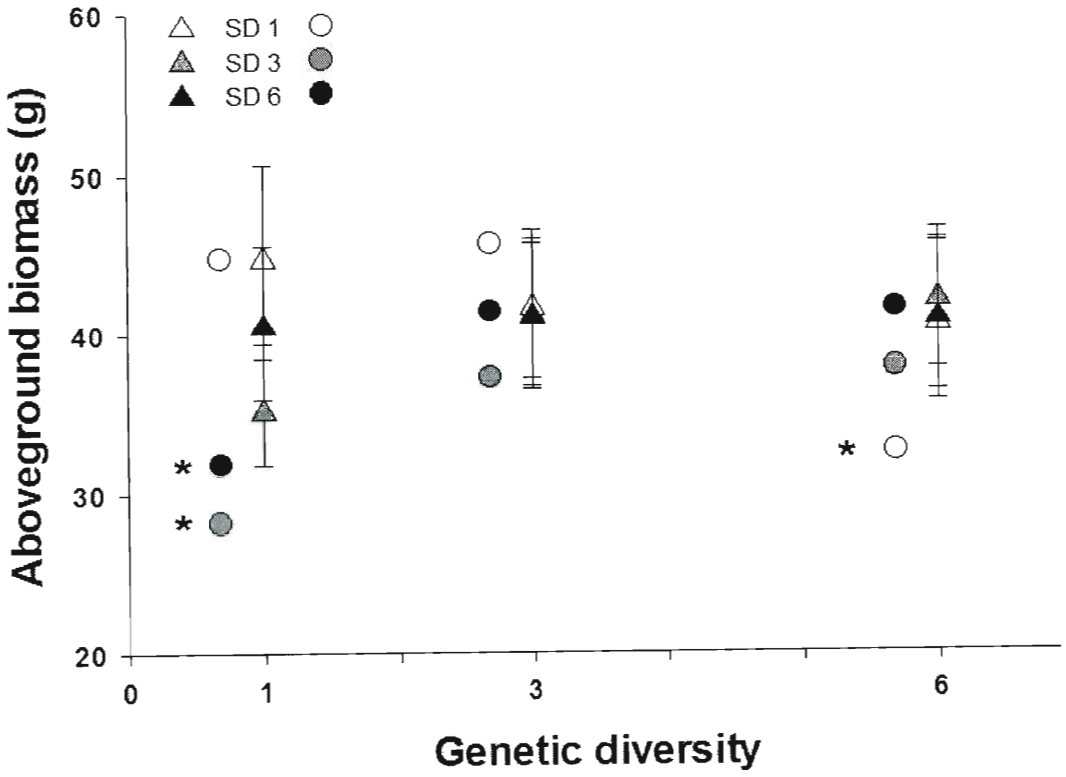


Figure 2.4 Additive expectations for aboveground biomass production versus experimental treatment means for species diversity treatments in crossed plots. Triangles are the additive expectation based on 10,000 Monte Carlo simulations surrounded by 95% confidence intervals. Actual treatment means are represented by circles and are slightly offset for visual clarity. Asterisks indicate values that are statistically significant from the additive expectation.

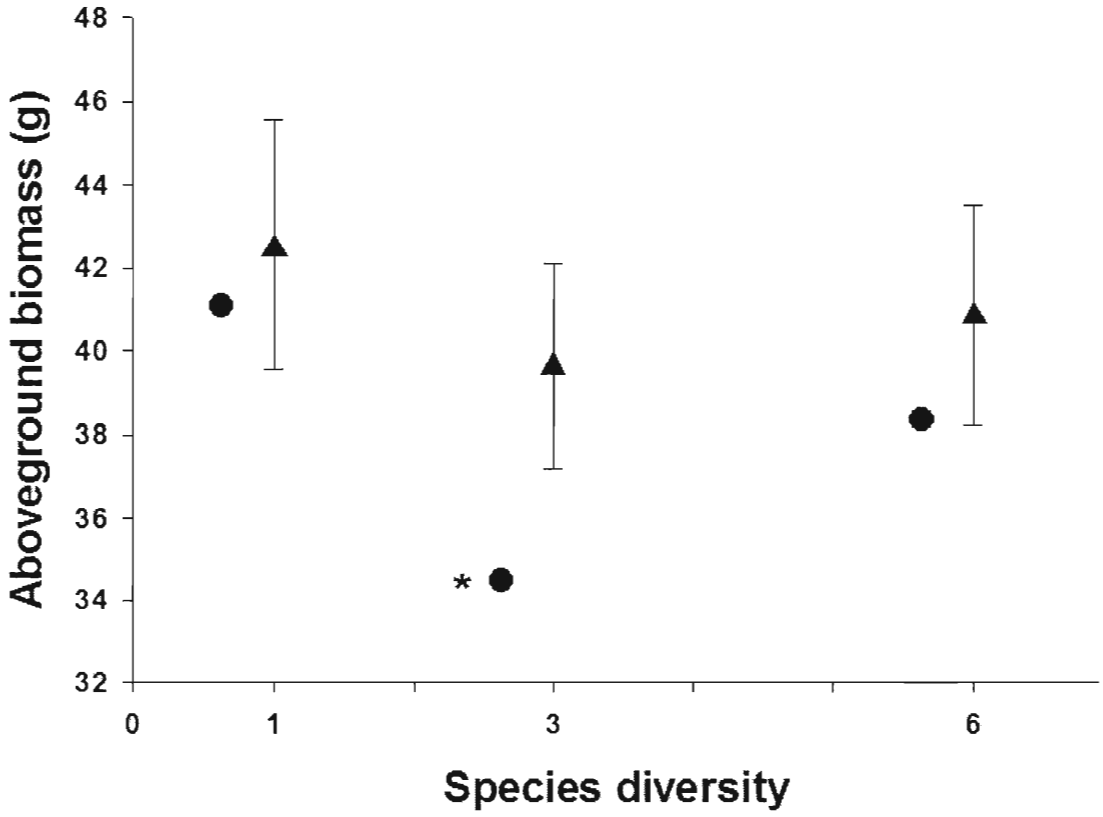
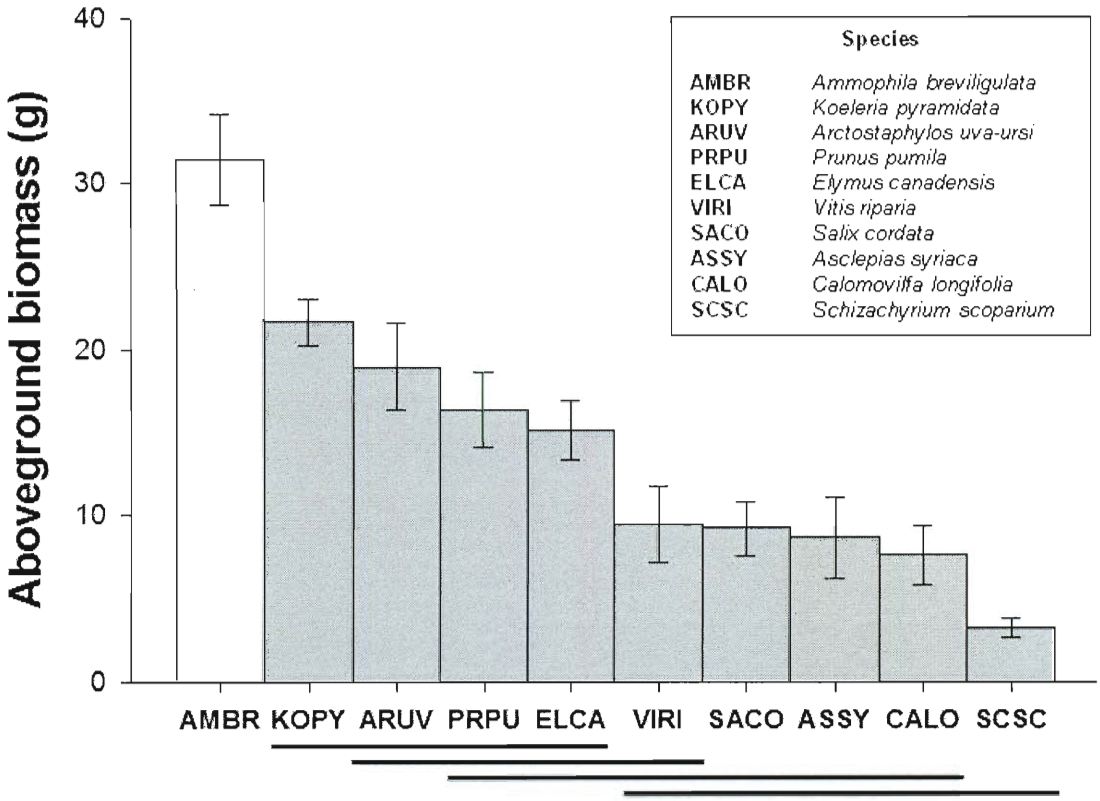


Figure 2.5 The average aboveground biomass produced per species in monoculture. *Ammophila breviligulata* produced significantly more biomass than all other species, but different populations of *A. breviligulata* did not produce statistically different amounts of biomass. Bars show means ± s.e. Horizontal lines below the species show the results of a Tukey's post-hoc analysis of species (excluding *Ammophila breviligulata*) biomass. Species sharing a line do not produce significantly different amounts of aboveground biomass.



2.9 APPENDIX A

To conduct genetic analyses of the *Ammophila breviligulata* populations, we collected material from 8 individuals in each of the 14 populations used in the experiment. Individual ramets were chosen haphazardly from the *A. breviligulata* populations. We harvested one leaf blade per individual and stored the samples in plastic sandwich bags. Bags were placed on ice in the field and stored at -80°C after overnight shipping to Rice University.

We extracted and purified DNA from each sample (112 total) using the QIAGEN (Valencia, CA, USA) DNeasy Plant DNA kit. Then, DNA was quantified using a Thermo Fisher Scientific (Wilmington, DE, USA) NanoDrop 2000 spectrophotometer. The three ISSR primers that we used were previously screened by Fant et al. (2008) for work on *A. breviligulata*. Several studies have found that three ISSR primers are usually sufficient to genotype every individual in a natural population (Wolfe et al. 1998a, 1998b, Esselman et al. 1999). Fant et al. (2008) chose these primers from a pool of 90 based on their reproducibility and the presence of polymorphic bands. For our samples, the primers (GA)8T, (GA)8C, and (CA)8G produced 13, 14, and 8 polymorphic bands, respectively. We prepared samples for PCR using Promega (Madison, WI, USA) PCR Mastermix. Each reaction contained 10 ng of genomic DNA, 1 mM primer, 1.5 mM MgCl2, 0.35 U Taq polymerase and 200 μM of dNTPs at a final volume of 20μL. PCR was performed in PTC-100 Programmable Thermal Cyclers (Bio-Rad, Hercules, CA, USA). Amplification conditions were as follows: one cycle of 94°C for 5 minutes, 35 cycles of 94°C for 45 seconds, 44°C for 45 seconds, 72°C for 2 minutes, followed by one cycle of 72°C for 10 minutes. Products were run on a 1.5% agarose gel. Banding patters

were visualized using SybrGreen (Applied Biosystems, Carlsbad, CA, USA) and recorded using the Kodak Photo ID program.

The resulting polymorphic bands for each individual were scored for presence/absence. These data were used to calculate pair-wise Sørensen similarity between individuals. Then, we tested whether similarity within populations was greater than similarities between populations using ANOSIM (PRIMER v6, Clarke and Gorley 2006). In short, ANOSIM calculates a test statistic, R, comparing rank similarities between and within a priori groups. Then, the significance of the test statistic is determined by comparing it to the distribution of the statistic when recomputed using 999 random permutations of the data.

Analysis of the banding patterns showed that no population was composed of only one genotype of *Ammophila breviligulata*. However, we did find that genetic similarity was greater within populations than between populations (Global R = 0.799, $P < 0.01$). Furthermore, each population significantly differed from each other at $P < 0.05$, except for populations 3 and 12 ($P = 0.12$), which were marginally different (Table 2.A1). Since populations are, for the most part, genetically distinct, we conclude that our genetic diversity treatment, which increases genetic diversity in *A. breviligulata* by increasing the number of populations in the community, is valid.

Table 2.A1 Results from ANOSIM showing the pair-wise genetic differences between populations of *Ammophila breviligulata*. Numbers (1-14) in the first row and first column represent separate *A. breviligulata* populations. Data shown are R statistics, which reflect the degree of dissimilarity between populations (low R = more similar, high R = less similar). Italicized R-values are not significant at $P < 0.05$.

	Pair-Wise Tests of Similarity between *Ammophila breviligulata* Populations												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
2	0.544												
3	1	0.804											
4	1	0.949	0.966										
5	0.380	0.556	1	0.96									
6	0.622	0.734	0.805	0.72	0.579								
7	0.778	0.789	0.926	0.997	0.717	0.847							
8	0.746	0.596	1	0.954	0.342	0.410	0.866						
9	0.691	0.483	0.965	0.949	0.697	0.604	0.807	0.597					
10	0.998	1	1	1	1	0.940	1	1	0.997				
11	0.861	0.654	1	0.995	0.546	0.558	0.892	0.707	0.538	0.999			
12	0.317	0.257	*0.135*	0.694	0.740	0.596	0.701	0.694	0.390	0.945	0.565		
13	1	1	1	1	0.982	0.548	1	0.946	1	1	1	0.920	
14	1	1	1	1	0.698	0.460	0.982	0.769	0.984	1	1	0.898	1

2.10 APPENDIX B

To survey aboveground biomass nondestructively, allometric equations relating plant traits to aboveground biomass were derived for each species in the experiment. For species propagated in nurseries, measurements were collected from plants propagated for the experiment, and planted in non-experimental monocultures next to the common garden. For species directly transplanted into the experimental plots (*Calomovilfa longifolia* and *Koeleria pyramidata*), measurements were collected from plants in the population from which the experimental plants were derived. A minimum of 16 plants were collected over multiple years from a range of different sizes to ensure that the experimental plants were within the range of the values used to construct the allometric equations (Table 2.B1). To mimic experimental measurements, all plant traits were measured in the field prior to harvesting the plants. Then, the measured plants were collected, dried to a constant mass, and weighed. Correlations relating plant traits to mass were performed to determine the best predictors of aboveground biomass (Proc REG, SAS Institute 2009). Since plant mass would be zero when measurements were zero, the intercept was forced through the origin. The resulting allometric equations explained at least 88% of the variation in plant mass for all species except *Asclepias syriaca* (Table 2.B1).

Table 2. B1 Allometric equations constructed for each species. The corresponding *F*, *P*, and r^2 values are derived from regressions through the origin relating plant traits to plant weight. The range is the minimum and maximum weight of plants used to construct the correlations.

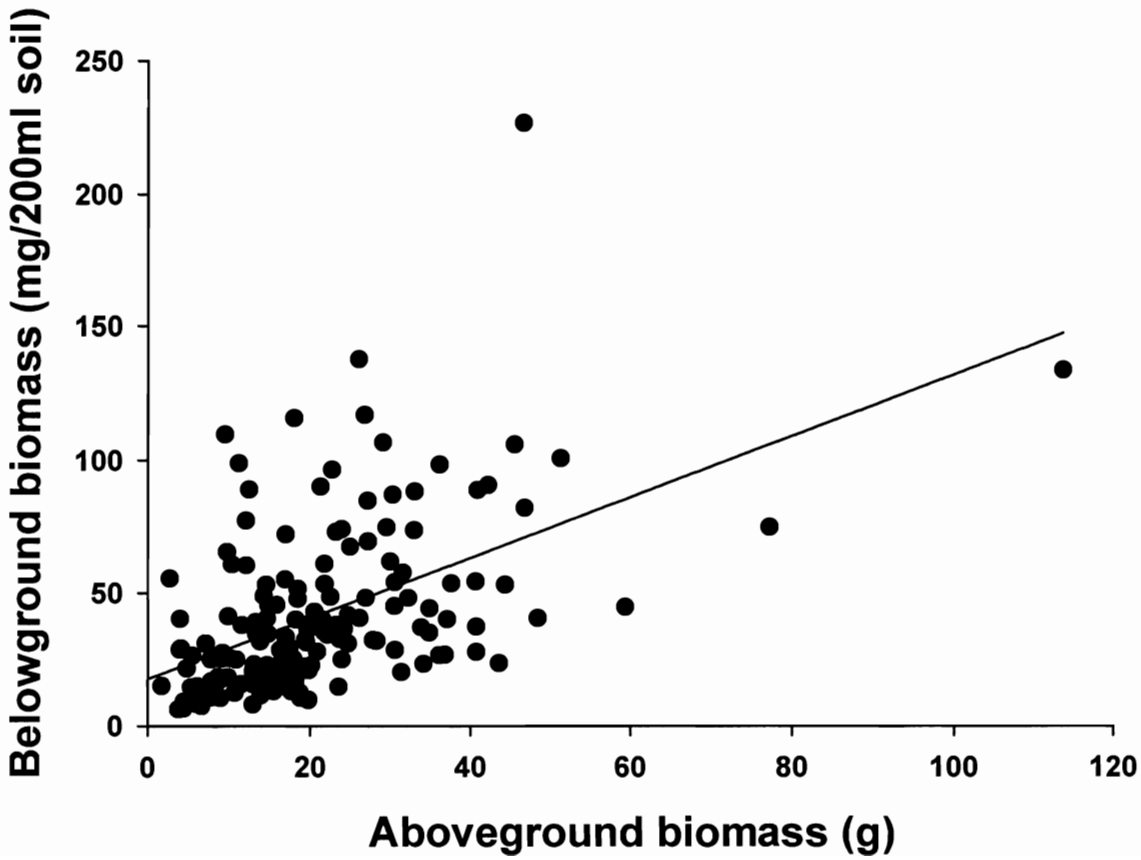
Allometric Equations						
Species	**Equation**	**N**	***F***	***P***	r^2	***Range (g)***
AMBR	y = 0.00723×MaxHeight×TillerNumber	28	773.83	<0.0001	0.97	0.81-8.23
ARUV	y = 0.02648×MaxHeight×BranchNumber	25	341.54	<0.0001	0.93	0.43-2.35
ASSY	y = 0.25188×StemDiameter	24	79.69	<0.0001	0.78	0.02-1.95
CALO	y = 0.00379×MaxHeight×TillerNumber×MaxTillerDiameter	25	325.98	<0.0001	0.93	0.24-6.92
ELCA	y = 0.00825×MaxHeight×MaxTillerDiameter	25	369.99	<0.0001	0.94	0.06-0.59
KOPY	y = 0.00368×MaxHeight×TillerNumber	21	146.83	<0.0001	0.88	0.09-3.17
PRPU	y = 0.01669×MaxHeight×StemDiameter	25	710.20	<0.0001	0.97	0.10-1.61
SACO	y = 0.03354×MaxHeight	16	254.80	<0.0001	0.94	0.07-1.88
SCSC	y = 0.00644×LeafNumber	25	274.83	<0.0001	0.92	0.01-0.29
VIRI	y = 0.02229×MaxHeight×StemDiameter	26	305.66	<0.0001	0.92	0.04-1.81

2.11 APPENDIX C

Table 2.C1 Results from general linear models testing how plant diversity influenced average belowground biomass production. "Crossed GD x SD" includes plots with both levels of diversity manipulated. "GD only" and "SD only" include plots where only genetic diversity or species diversity were manipulated. The effects of G ID and S ID were analyzed using monoculture plots.

Belowground Biomass				
		df	*F*	*P*
Crossed GD x SD	**Genetic diversity**	1	0.25	0.6165
	Species diversity	1	0.54	0.4647
	GD x SD	1	1.06	0.3082
	Error	59		
GD only	**Genetic diversity**	1	2.42	0.1255
	Error	54		
SD only	**Species diversity**	1	0.12	0.7266
	Error	39		
G ID	**Genetic identity**	13	1.00	0.4763
	Error	28		
S ID	**Species identity**	8	1.07	0.4256
	Error	18		

Figure 2.C1 The correlation between aboveground and belowground biomass for all plots. Aboveground biomass is averaged over the season and belowground biomass is the average of two 200mL subsamples. Each plot is represented by a solid circle.



Chapter 3

Plant Genetic Diversity Trumps Species Diversity in Structuring an Arthropod Community

Biodiversity is being lost at a rapid rate, leading to much interest in the role biodiversity plays in structuring communities. For example, losses in plant diversity may cascade to influence higher trophic levels. Experimental tests have shown that plant species diversity and genetic diversity within plant species influence arthropod community structure. However, the majority of these studies have been conducted in separate systems, so the relative importance of plant species diversity and genetic diversity is currently unresolved. Furthermore, potential interactions between the two levels of diversity, which likely occur in natural systems, have not been investigated. Here, in a common garden experiment where we factorially and independently manipulated plant species diversity and genetic diversity within a dominant species, we tested how arthropod communities responded to changes in plant diversity. Overall, we found that genetic diversity within the dominant plant species, *Ammophila breviligulata*, more strongly influenced arthropod communities than plant species diversity. In plots containing both the dominant species and the other plant species, arthropod abundance and morphospecies richness peaked at the highest level of genetic diversity. These effects were driven by positive non-additive effects of diversity, suggesting that arthropods respond to emergent properties of diverse plant communities. Genetic diversity also significantly altered the overall composition of the arthropod community. In plots where genetic diversity within *A. breviligulata* was manipulated in the absence of other species, only arthropod morphospecies richness was affected, suggesting a stronger

role for genetic diversity in a more complex (non-monoculture) plant community. In contrast, arthropod communities did not respond to plant species diversity in either the factorial or independent diversity manipulations. In addition to showing that genetic diversity within a dominant plant species can have large effects on arthropod community composition, these results suggest that understanding how species diversity and genetic diversity interact to influence community structure may be critically important for predicting the consequences of biodiversity loss.

3.1 INTRODUCTION

In response to rapid rates of biodiversity loss (Pimm et al. 1995, Chapin et al. 2000), a large body of ecological research has documented the consequences of biodiversity for ecosystem functioning (reviewed by: Loreau et al. 2002, Hooper et al. 2005, Hughes 2008). Most experimental work to date has investigated how reductions in plant diversity influence terrestrial ecosystems, and has found that, generally, ecosystem function declines with decreasing biodiversity (meta-analyses: Balvanera et al. 2006, Cardinale et al. 2006). For example, experimental reductions in plant diversity have revealed decreases in net primary productivity (Tilman et al. 1996, Tilman et al. 2001, Crutsinger et al. 2006), nutrient cycling (Tilman et al. 1996, Knops et al. 2001, Fornara and Tilman 2008), and the stability of both communities (van Ruijven et al. 2003, Crutsinger et al. 2008b) and ecosystem processes (Tilman 1996, Hughes and Stachowicz 2004, Reusch et al. 2005, Tilman et al. 2006). Since plants provide the basis for terrestrial food webs, the consequences of diversity loss can cascade to higher trophic levels (Hutchinson 1959, Hunter and Price 1992), potentially leading to greater losses in biodiversity.

Prior work has demonstrated that plant species diversity affects arthropod community structure. Generally, as plant species richness increases, the number of arthropod species increases (Siemann et al. 1998, Knops et al. 1999, Haddad et al. 2009). This relationship may arise via a number of non-mutually exclusive mechanisms. First, as plant diversity increases the diversity of resources available to arthropods grows, increasing the probability of including specialist herbivores (Hutchinson 1959, Strong et al. 1984). These increases could cascade to higher trophic levels, increasing the diversity of parasites and predators (Hunter and Price 1992, Siemann 1998). Effects of resource diversity on arthropod richness may be explained by additive effects of diversity. Additive effects occur when the response of arthropods to diverse plant communities can be predicted by the responses of arthropods to the component plant species in monoculture -- more plant species available as resources attracts more arthropod species specializing on those resources (sampling effect or selection effect, *sensu* Loreau and Hector 2001). Second, increased plant diversity also generally leads to greater primary productivity (Tilman 1996, Cardinale et al. 2006), which can provide a greater quantity of resources to arthropods, potentially increasing arthropod abundance (Root 1973). As the abundance of arthropod individuals increases, the probability of observing more arthropod species also increases (Srivastava and Lawton 1998, Gotelli and Colwell 2001). Third, increased plant diversity can enhance the structural complexity of the plant community, potentially attracting some arthropod species (Halaj et al. 2000) and altering arthropod community composition (Dennis et al. 1998). Changes in arthropod community composition with increased plant biomass or habitat complexity could reflect non-additive effects of diversity. Non-additive effects of diversity occur when the

response of the arthropod community cannot be predicted from the response of the arthropod species in the component plant monocultures. In this case, biomass and structural complexity are emergent properties of the diverse plant community; therefore, arthropod responses are non-additive in nature. In addition to changes in overall arthropod species richness, arthropods differing in feeding habit, mobility, and resource specialization can differentially respond to changes in plant diversity (Knops et al. 1999, Koricheva et al. 2000, Haddad et al. 2001), altering the overall composition of arthropod communities.

Just as plant species richness can influence arthropod community structure, genetic diversity within populations of plants can structure arthropod communities. For example, in populations of tall goldenrod (*Solidago altissima*) arthropod richness and abundance increased with increasing genetic diversity (Crutsinger et al. 2006, 2008a). These effects were driven by positive non-additive effects of diversity, possibly due to non-additive increases in biomass production in genetically diverse populations (Crutsinger et al. 2006, 2008a). Similarly, populations of evening primrose (*Oenothera biennis*) composed of eight genotypes hosted 18% more arthropod species than genetic monocultures and had increased abundances of predators and omnivores (Johnson et al. 2006). However, these responses were driven primarily by additive effects of diversity (Johnson et al. 2006).

While both plant species diversity and genetic diversity are documented to be important determinants of arthropod community structure, little is known about their relative importance. Only one study to date has directly compared the effects of plant species diversity and genetic diversity on arthropod communities in a single experiment

(Cook-Patton et al., *in press*). Cook-Patton et al. (*in press*) experimentally manipulated diversity by establishing monocultures and polycultures of old-field plant species and monocultures and polycultures of *Oenothera biennis* genotypes. They found that arthropod richness was greater in species polycultures relative to species monocultures and in genetic polycultures relative to genetic monocultures; however, the magnitude of the effect of genetic diversity was weaker than the effect of species diversity. This result makes intuitive sense – mechanisms underlying positive diversity responses rely on phenotypic variability among individual species or genotypes (e.g. variation in resources or plant architecture), and variation among genotypes is expected to be lower than variation among species. However, results from independent experiments suggest that sometimes genetic diversity can have effects that are as strong or stronger than the effects of species diversity (Crutsinger et al. 2006, Johnson et al. 2006).

The conditions that promote strong effects of genetic diversity within a species remain unclear, but may include the relative abundance and diversity of other species in the community. While prior studies contribute to understanding how arthropod communities are structured, they fail to examine the potentially important interactions between species and genotypes within species that occur in natural communities. For example, in a community composed of two plant species, arthropods may respond quite differently when each plant species is represented by many genotypes versus a single genotype because of the greater intraspecific variation. Furthermore, these effects may be non-additive if arthropods respond to community-level properties (such as biomass production, resource quality, or habitat complexity) that are altered when several genotypes of each species are grown together. Understanding how these interactions

influence arthropod community structure is critical for predicting how losses in biodiversity will cascade to influence higher trophic levels.

Utilizing a common garden where we manipulated both plant species diversity and genetic diversity in a dominant species, we addressed how plant species diversity and genetic diversity independently and interactively influenced arthropod communities. Specifically, we tested: (1) What is the relative importance of plant species diversity and genetic diversity for the structure of arthropod communities? (2) Do interactions between plant species diversity and genetic diversity alter arthropod community structure?

3.2 METHODS

Study system – This experiment was conducted in the dune system surrounding Lake Michigan at Sleeping Bear Dunes National Lakeshore (44° 43.689' N, 86° 07.369' W). Great Lakes sand dunes support plant communities of relatively low species richness (1-5 species/m^2, Crawford, unpublished data, Cowles 1899), making this an ideal ecosystem for realistic, yet feasible manipulations of species diversity. Dune plant species comprise a variety of functional types, including several grasses, woody species, and forbs. The dominant plant species, *Ammophila breviligulata* (American beachgrass), grows primarily via ramets and acts as an ecosystem engineer by stabilizing sand during primary succession, which then allows other plants to colonize (Olson 1958, Cheplick 2005). Natural populations of *A. breviligulata* are typically composed of 1-5 genotypes per m^2 (Fant et al. 2008).

Dune arthropod communities have been shown to be sensitive to changes in plant community composition (Marshall et al. 2008, Baskett et al., *in press*). Arthropods also play an important role in influencing plant succession in this ecosystem. For example,

herbivory by the beetle *Altica subplicata* on *Salix cordata* caused significant shifts in the abundance of several common plant species (Bach 2001). Like many terrestrial ecosystems at the land-water interface, dune systems typically support more predatory arthropods than herbivores, with a predator to herbivore ratio of 6:1 (Crawford, *unpublished data*, see also Polis and Hurd 1995, Gratton et al. 2008). Abundant predators include tiger beetles (Coleoptera: Cicindelidae) and spiders (class Araneae). The most common herbivores are generally aphids (Hemiptera:Aphidoidea) and grasshoppers (suborder Caelifera). Some arthropods spend only a portion of their life-cycle on land, yet play important roles in the terrestrial ecosystem. For example, midge adults emerge from the Great Lakes in pulses during mating. While several lake-emergent midges do not feed as adults, they do serve as an important source of food for terrestrial animals and augment the nutrient-poor soil via decomposition (Smith et al. 2007, Gratton 2008).

Plant material – We collected all plant material from Sleeping Bear Dunes National Lakeshore during July 2007. *Ammophila breviligulata* ramets were collected from 14 populations that were separated by at least 1km and grown at a commercial nursery that specializes in propagation of *A. breviligulata* for ecological restoration (VansPines Nursery, Holland, Michigan, USA). Nine other plant species were collected for the manipulation of species diversity. These included four grasses (*Calomovilfa longifolia*, *Elymus canadensis*, *Koeleria pyramidata*, and *Schizachyrium scoparium*), four woody species (*Arctostaphylos uva-ursi*, *Prunus pumila*, *Vitis riparia*, and *Salix cordata*) and a forb (*Asclepias syriaca*). The woody species were propagated from cuttings collected from 3-5 mature individuals. For the other species, material was collected from a single population. All cuttings and seeds were propagated at a commercial nursery in

Michigan (Richey Nursery Company, LLC, Spring Lake, MI, USA), with the exception of *C. longifolia* and *K. pyramidata,* which were collected near the common garden and directly transplanted into the plots. Two of the plants included in the study host specialist herbivores. *Salix exigua* hosts two specialist Chrysomelids, *Altica subplicata* and *Disonycha alternate,* and *Asclepias syriaca* hosts a Cerambycid, *Tetraopes spp.*, and larvae of the Monarch butterfly, *Danaus plexippus*. Several arthropod species can reach high abundances on the dunes, including the *Salix* specialist beetles, tent caterpillars (Lepidoptera: Lasiocampidae), and midges of the superfamily Chironomoidae.

Characterizing genetic diversity – To ensure the validity of our genetic diversity treatment, we examined the genetic diversity within and among the populations of *A. breviligulata* using intersimple sequence repeat (ISSR) markers. Genetic variation among populations was greater than genetic variation within populations (Crawford & Rudgers, *in review*). Additionally, all populations except two (3 and 12) significantly differed in their banding patterns. These two populations never occurred together in treatments with three populations of *A. breviligulata* and only occurred together in 2 of 28 plots with six populations of *A. breviligulata.* These results confirmed that by increasing the number of populations in a community, we increased genetic diversity within *A. breviligulata.* For additional details on the molecular analysis, see Crawford and Rudgers, *in review*.

Common garden – The common garden was established at a site where the National Park Service demolished homes in 2004 to perform a restoration of the dune habitat. Few plants had colonized the area since demolition ($<0.25/m^2$); non-native species were manually removed and native species were relocated prior to plot

establishment. Due to differences among species in optimal planting time, we established the experiment in three phases. We planted *Ammophila breviligulata* in mid-October 2007, because local land managers reported greater success with fall plantings. The following June, we planted the woody species, and we planted the remaining species in July. Plots were watered during the summer of 2008 to promote establishment, and were weeded monthly during the growing seasons to maintain diversity treatments.

Genetic diversity x species diversity manipulation (crossed plots) – To examine the interactive effects of plant species diversity and genetic diversity on arthropod communities, we factorially crossed three levels of species diversity (1, 3, or 6 species) with three levels of genetic diversity within *A. breviligulata* (1, 3, or 6 populations) (Figure 3.1). We established treatments in 1.5m X 1.5m plots at a density of 24 plants per plot, comprised of 12 individuals of *A. breviligulata* and 12 individuals of the other species, to create a realistic density and composition for this community (Figure 3.1). Therefore, the species diversity treatments describe the species richness of the plot, excluding *A. breviligulata*. Within the species diversity and genetic diversity treatments, we planted equal numbers of individuals for each species/population. For example, in a plot with a treatment combination of 3 species and 6 populations, we planted 4 individuals of each of the 3 species other than *A. breviligulata* and 2 individuals of each *A. breviligulata* population. Each treatment combination was replicated 7 times for a total of 63 plots.

To minimize the potential for quasi-replication - the replication of a specific community in the highest diversity treatment that confounds diversity effects with community composition effects (Huston & McBride 2002) - *A. breviligulata* populations

were selected randomly from a pool of 14, and other species were selected randomly from a pool of nine. However, to avoid increases in the similarity of communities at high diversity levels, random combinations were chosen to maximize dissimilarity within treatments. For example, replicates containing 6 populations of *A. breviligulata* and 3 species were allowed to have 2 of the 14 populations in common and 2 of the 9 species in common. If a treatment replicate deviated from these stipulations, the replicate was discarded, and a new replicate was randomly generated.

Independent diversity manipulations and monocultures (independent plots) – In our simultaneous manipulations of diversity, plots contained equal numbers of both the dominant and non-dominant species. However, we expected that diversity effects on arthropods may differ between communities with and without the dominant plant. Thus, we established additional plots that manipulated only species diversity or genetic diversity (1, 3, or 6 species/populations) in *A. breviligulata* to measure their independent effects (Figure 3.1). Plots with 3 or 6 species/populations were established at the same size and density as the crossed diversity plots. Plots containing only one species or one population (monocultures) were established to obtain individual effects on arthropod communities and allow the partitioning of additive versus non-additive effects. Due to space and labor limitations, monocultures were planted at the same density as individuals in the diversity plots, but with 12 individuals per plot. Each population monoculture (14 total) and species monoculture (9 total) was replicated three times, and independent diversity plots were replicated seven times, for a total of 97 plots. As with the species diversity x genetic diversity plots, the potential for quasi-replication was minimized, and dissimilarity within treatment combinations was maximized.

Arthropod sampling – We sampled arthropods throughout the 2010 growing season using pitfall traps. Arthropod abundances were very low, so other methods that survey arthropods during a time interval of a few minutes (sweep netting and visual surveys) did not provide the necessary power to detect how plant diversity influenced arthropod community structure. Pitfall traps were constructed from 50ml centrifuge tubes. Tubes were filled with 20mL of water, and one drop of fragrance free soap was added to break the surface tension of the water. We placed one tube in the center of each plot, flush with the ground. Pitfall traps were left in place for five days to capture arthropods. After gathering the tubes, we collected all individuals in each trap, identified them to morphospecies, and preserved them in 70% ethanol. All morphospecies were identified to order, except three individuals of the subphylum Myripoda, and 80% were identified to family. Based on order and family identifications, arthropods were assigned to one of six functional groups based on adult feeding strategies: predators, parasitoids, herbivores, non-feeding adults, detritivores, and omnivores. Family identifications and assignment to functional groups were made using descriptions in Arnett (2000), Triplehorn and Johnson (2005), and Marshall (2006). Pitfall trapping was repeated for each month of the growing season (May-August), but not more often to assure that arthropod populations were not depleted. Pitfall traps captured both ground-dwelling arthropods and aerial arthropods (35% of collected individuals were in the order Diptera), and communities were similar in composition to visually surveyed arthropod communities (Crawford, *unpublished data*).

Statistical analyses: During the course of the experiment, some plant mortality occurred. Models incorporating mortality as realized diversity did not differ qualitatively

from models using the initially planted diversity, so the latter models are presented for ease of interpretation. Morphospecies that were only collected once during the course of the field season (25 total) were excluded from all analyses.

Statistical analyses: Crossed plots

Community composition -- We tested how plant diversity and time influenced total community composition using a permutational multivariate analysis of variance (PERMANOVA) (PRIMER software, Anderson et al. 2008), a nonparametric test for multivariate data. In short, PERMANOVA performs an analysis that is analogous to M/ANOVA using multivariate data. From a similarity matrix, distance-based pseudo-F statistics are calculated based on expectations of mean squares, and P-values are derived using a permutation procedure (Anderson et al. 2008). Before analyses, data were transformed by adding one to each value so that similarities could be calculated between samples with no observed arthropods (4 plots in July and 1 plot in August) (Clarke and Gorley, 2006). PERMANOVA models incorporating square-root transformed data, which reduces the influence of highly abundant species (Clarke and Warwick 2001), provided qualitatively similar results. We used Bray-Curtis similarity to calculate pair-wise similarity between samples (following McCune and Grace 2002), and ran 9,999 permutations. For crossed plots, our full PERMANOVA model included plant species diversity, genetic diversity, time, plot (nested in genetic diversity and species diversity), and all possible interactions. Data met the assumption of exchangibility of samples (Anderson et al. 2008). Significant treatment effects were followed by pair-wise tests between treatment levels (comparable to t-tests).

To visualize significant PERMANOVA results, we performed ordination of the data using non-metric multidimensional scaling (NMS) in PRIMER (Clarke and Gorley 2006). NMS creates a graphical representation of rank similarity of the experimental plots, such that plots closer in space are more similar than plots distant in space. NMS was conducted on the similarity matrices constructed for PERMANOVA. To ensure that stress values were equal across runs (reflective of the global minimum instead of local minima), NMS was performed at least 3 times with 999 restarts (Clarke and Warwick 2001). To determine which morphospecies were driving ordination patterns, we examined how much of the variation among groups was explained by each morphospecies using a "similarity percentages routine" (SIMPER) (PRIMER, Clarke and Gorley 2006), which decomposed average Bray-Curtis dissimilarities into the percentage contributions from each morphospecies. For morphospecies that explained more than 10% of the variation among treatments, we tested how the abundance of the morphospecies was affected by the treatment using mixed models, described below (Proc MIXED, SAS Institute 2009).

Arthropod abundance, richness, and evenness – In the crossed plots (plots simultaneously manipulating species diversity and genetic diversity), we tested for the effects of plant diversity and time on arthropod abundance, richness, and evenness using repeated measures mixed models. Models treating species diversity and genetic diversity as categorical predictor variables rather than continuous predictor variables had a better fit based on AIC, so plant species diversity and genetic diversity were treated as categorical predictor variables in the models. The full models included the fixed effects of plant species diversity, genetic diversity, time, and all possible interactions (Proc

MIXED, SAS Institute 2009). Results were tested over the plot-level error. An unstructured covariance model was chosen based on AIC, and standard errors and *F*-statistics were KR corrected (Kenward and Roger 1997). Either no arthropods or only one individual was collected from 10 plots in July and one plot in August. Evenness could not be calculated for these plots, so they were excluded from the analyses. Data met assumptions of normality and homogeneity of variances.

It is likely that arthropod abundance increases with plant biomass, independent of any effects of plant species diversity. In the crossed plots, plant diversity significantly affected primary productivity (Crawford and Rudgers, *in review*). To see if differential biomass influenced arthropod communities, we tested for a correlation between arthropod abundance and plot-level biomass (Proc REG, SAS Institute 2009).

Response of herbivores and predators – We also examined how herbivore and predator functional groups responded to our diversity treatments. The omnivore, detritivore, and non-feeding adult functional groups were dominated by one or two morphospecies, so responses within these groups were not analyzed. Furthermore, only a small fraction of the arthropod community (~0.5%) was parasitoids, so this group was also not analyzed. For herbivores and predators, both richness and abundance were analyzed using the mixed models described above, as was the herbivore to predator ratio (Proc MIXED, SAS Institute 2009). Thirty-two of the 100 morphospecies, representing 30% of collected individuals, were not identified to a fine enough resolution for assignment to a trophic group and were excluded from the analyses.

Additive versus non-additive effects – When statistically significant main effects of diversity were detected, we tested whether the effect was driven by the composition of

the plant community (additive effects of diversity) or by emergent effects of diversity (non-additive diversity effects). Monte Carlo simulations used to test for non-additivity require data for individual plants in monoculture (Johnson et al. 2006, Crutsinger *et al.* 2006, Crawford & Whitney 2010), but we collected plot-level data using the pitfall traps. Therefore, we calculated the net biodiversity effect by utilizing a modified version of the method created by Loreau and Hector (2001), and used previously by Johnson et al. (2006). First, the average response of arthropods throughout the field season in each monoculture plot was divided by the number of plants (12), yielding a per plant estimate of arthropod responses. Then, the per-plant value was averaged for each species and genotype over the three monoculture plots, yielding an average per-plant response for each species and genotype. Monoculture plots contained half the number of plants relative to diversity plots; thus, before calculating the additive expectations, monoculture per-plant averages were halved. We then used these values to create a dataset for expected (additive) responses by matching the composition (species composition and genotypic composition) of each of the experimental diversity plots with the expected per-plant monoculture values. To compare the observed and expected (additive) values, we performed nonparametric ANOVA blocked by plot, with the significant diversity factor(s), dataset (observed versus expected), and all possible interactions. The distributions of the observed and expected abundances (and residuals) were non-normal. Therefore, we used distribution-free randomization tests with 9,999 iterations to evaluate treatment effects (Edgington, 1987; Manly, 1991). A randomization test determines a *p*-value by comparing an observed test statistic (here, the *p*-value) with a distribution of the test statistic that is expected under the null hypothesis that the treatment has no effect. We

applied a randomization test equivalent of a general linear model by encompassing Proc MIXED code within a SAS v. 9.1 (SAS Institute, Cary, NC, USA) randomization macro program (Cassell, 2002). A significant dataset x diversity effect would indicate that the deviation from the additive expectation was dependent on the level of plant diversity.

Statistical analyses: Independent plots

Community composition – To test how diversity influenced arthropod community composition in the independent diversity manipulations, we used PERMANOVA with plant species diversity or genetic diversity, time, plot (nested in either species diversity or genetic diversity), and all possible interactions. Significant effects were visualized using NMS ordination, treated as described above.

Arthropod abundance, richness, and evenness and additive versus non-additive effects – In the independent diversity manipulations (plots containing only species diversity or genetic diversity manipulations), we tested how diversity influenced arthropod abundance, richness, evenness, and herbivore and predators using repeated measures mixed models. The full models included the fixed effect of either plant species diversity or genetic diversity, along with time and the time by diversity interaction (Proc MIXED, SAS Institute 2009). Other than the statistical model, analysis methods were the same as for the crossed plots, including the calculation of additive versus non-additive effects. Independently, neither plant species diversity nor genetic diversity influenced plant biomass production (Crawford and Rudgers, *in review)*. Therefore, we did not test for a correlation between arthropod abundance and plant biomass.

3.3 RESULTS

At the end of the field season, 13,608 individual arthropods representing 17 orders, over 70 families, and 100 morphospecies were collected. The families with the most individuals included ants, Formicidae (>3,300 individuals), biting midges, Ceratopogonidae (>2,700 individuals), ant-like flower beetles, Anthicidae (>1,700 individuals), and non-biting midges, Chironomidae (> 1,100 individuals). For a full list of morphospecies along with their identifications, functional group assignments, and abundances, please see Appendix A.

Crossed Plots

Community composition -- Plant genetic diversity significantly influenced the overall composition of arthropod communities, and these effects shifted through time, as indicated by a significant time x genetic diversity interaction (Table 3.1, Figure 3.2). In May and June, arthropod communities in plots with six populations of *A. breviligulata* were significantly different from communities in plots with either one population (May: $t = 2.2748$, $P = 0.0011$; June: $t = 1.9872$, $P = 0.0021$) or three populations of *A. breviligulata* (May: $t = 2.4391$, $P = 0.0014$; June: $t = 2.1699$, $P = 0.0018$). In July, there was no significant effect of genetic diversity on arthropod community structure, and in August, communities differed between plots with one or three populations ($t = 1.4652$, $P = 0.0269$). Across all time points, communities with six populations of *A. breviligulata* significantly differed in their composition relative to communities with either one ($t = 1.9677$, $P = 0.0021$) or three ($t = 2.1184$, $P = 0.0024$) populations. In contrast to the significant effect of genetic diversity, species diversity did not affect overall arthropod composition (Table 3.1).

The significant main effect of genetic diversity was driven by five of the most abundant morphospecies, which together explained 68% of the dissimilarity in arthropod community composition between plant communities with 3 and 6 populations of *A. breviligulata* and 66% of the variation between communities with 1 and 6 populations. For both comparisons, a biting midge (Diptera: Ceratopogonidae, one of two morphospecies in this family) explained >20% of the dissimilarity, and an ant (Hymenoptera: Formicidae, one of two morphospecies in this family) explained another 17% of the dissimilarity. Three additional morphospecies -- an ant-like flower beetle (Coleoptera: Anthicidae, only morphospecies in this family), an orbatid mite (Acari: Orbatidae, only morphospecies in this family), and a non-biting midge (Diptera: Chironomidae) – each explained about 10% of the dissimilarity. Throughout the field season, declines in the numbers of the two midges and the ant-like flower beetle caused significant shifts in community structure, with these three species explaining 50% of the dissimilarity between May and August.

Three of the five morphospecies driving differences in arthropod community structure among genetic diversity treatments increased in abundance with increased genetic diversity. Specifically, the abundance of orbatid mites was ~150% greater in communities with six populations of *A. breviligulata* relative to communities with three populations, which had the lowest total abundance of orbatid mites ($F_{2,54} = 3.52$, $P = 0.0364$). The abundance of the non-biting midge morphospecies was ~50% greater in plant communities with six populations of *A. breviligulata* relative to communities with three populations ($F_{2,54} = 4.05$, $P = 0.0230$), and the abundance of the Ceratopogonid midge morphospecies was 100% greater in plant communities containing six populations

of *A. breviligulata* versus communities containing either one or three populations ($F_{2,54}$ = 8.76, P = 0.0005).

Arthropod abundance, richness, and evenness -- In crossed plots, which contained both *A. breviligulata* and other species, arthropod abundance was significantly affected by genetic diversity (Table 3.1, Figure 3.3). Plant communities with six populations of *A. breviligulata* hosted almost 30% more arthropods than communities with three populations (Tukey's HSD P = 0.05), but arthropod abundance did not significantly differ between communities with one versus six (Tukey's HSD P = 0.16) or one versus three (Tukey's HSD P = 0.85) populations of *A. breviligulata*. Arthropod abundance was not correlated with community-level plant biomass ($F_{1,61}$ = 0.80, P = 0.3758), indicating that differences in arthropod abundance were not driven by diversity-mediated differences in plant biomass. Arthropod abundance peaked in June, averaging 40 individuals per plot, and was lowest in July, averaging only 6.5 individuals per plot, as evidenced by a significant effect of time (Table 3.1). As with arthropod composition, the effect of genetic diversity on arthropod abundance varied through time, with the largest difference between diversity treatments in May (Table 3.1). There was neither a significant main effect of species diversity on abundance nor a significant interactive effect between species diversity and genetic diversity (Table 3.1).

There was a strong trend for genetic diversity in crossed plots to significantly affect arthropod morphospecies richness (Table 3.1). Morphospecies richness was 16% greater in communities with 6 populations of *A. breviligulata* than plots with 3 populations (Tukey's HSD P = 0.05), but did not significantly differ between communities with one and six (Tukey's HSD P = 0.24) or one and three (Tukey's HSD P

= 0.69) populations (Figure 3.4A). Morphospecies richness was positively correlated with arthropod abundance ($F_{1,61} = 24.19$, $P < 0.0001$, $r = 0.49$), so it is likely that increased abundances are responsible for at least part of the significant effect of genetic diversity on morphospecies richness. Arthropod community evenness was not influenced by plant diversity in crossed plots (Table 3.1). For a full breakdown of how the abundance of each morphospecies responded to genetic diversity in the crossed plots, please see Appendix A, Table 3.A2.

Response of herbivores and predators – In the crossed plots, neither plant species diversity nor genetic diversity influenced abundance or richness within the herbivore and predator functional groups or the herbivore abundance to predator abundance ratio (Appendix B, Table 3.B1).

Additive versus non-additive effects -- The effect of genetic diversity on arthropod abundance was driven by a significant non-additive effect of diversity (Figure 3.3). When the actual experimental abundances were compared to the additive expectation, there was a significant dataset (observed versus expected) x genetic diversity interaction ($P < 0.0299$). When six populations of *A. breviligulata* were present, there were 43% more individual arthropods than expected (Tukey's HSD $P < 0.0001$). There was also a non-additive effect for arthropod morphospecies richness ($P = 0.0380$), with 27% more morphospecies present than expected for communities containing six populations of *A. breviligulata* (Figure 3.4A, Tukey's HSD $P < 0.0001$).

Independent plots

Community composition -- Neither species diversity nor genetic diversity significantly affected overall arthropod community composition when they were manipulated independently (Table 3.1).

Arthropod abundance, richness, and evenness – In contrast to crossed plots, where both the dominant plant species, *A. breviligulata*, and other plant species were present, arthropod abundance did not respond to independent manipulations of either species diversity or genetic diversity (Table 3.1). However, arthropod morphospecies richness did respond to genetic diversity in the independent plots (Table 3.1, Figure 3.4B), but somewhat differently than in the crossed plots. Morphospecies richness was still lowest with three populations of *A. breviligulata*, 19% lower than when six populations were present (Tukey's HSD $P = 0.05$); however, arthropod richness for three populations was also 15% lower than when one population was present (Tukey's HSD $P = 0.05$). There was no effect of plant species diversity on arthropod morphospecies richness (Table 3.1). Arthropod community evenness was not affected by plant diversity (Table 3.1). For a full breakdown of how the abundance of each morphospecies responded to genetic diversity in the independent plots, please see Appendix A, Table 3.A3.

Response of herbivores and predators – In contrast to plots where plant species diversity and genetic diversity were factorially manipulated, plant species diversity did significantly influence the herbivore abundance (Appendix B, Table 3.B1). Plant communities containing three species other than *A. breviligulata* contained the least herbivore individuals, 0.36±0.22, whereas communities with one other species contained

1.11±0.11 and communities with six other species contained 1.07±0.22. This led to a significantly lower herbivore to predator ratio in these treatments (Appendix B, Table 3.B1). Plant communities with three species other than *A. breviligulata* contained, on average, 0.19±0.24 herbivores for every predator, while communities with one other species contained 0.70±0.12 and communities with six other species contained 1.11±0.24. Neither plant species diversity nor genetic diversity independently influenced predator abundance or richness or the richness of herbivores.

Additive versus non-additive effects – The significant effect of genetic diversity on arthropod morphospecies richness in the independent diversity manipulation was driven by a negative non-additive effect of diversity (Figure 3.4B, data set (observed vs. expected) by genetic diversity interaction: $P = 0.0057$). Specifically, when three populations of *A. breviligulata* were present, the observed number of morphospecies was 14% lower than expected additively (Tukey's HSD $P = 0.02$).

3.4 DISCUSSION

We found that genetic diversity within the dominant plant species, *Ammophila breviligulata*, played a larger role in structuring arthropod communities than plant species diversity. Plant species diversity significantly influenced the number of herbivores present in plant communities when the dominant species was not present, altering the herbivore to predator ratio in these treatments. In contrast, plant genetic diversity influenced arthropod abundance, morphospecies richness, and community composition. In plant communities where both the dominant species and other species were present (crossed plots), arthropod abundance peaked at the highest level of genetic diversity, there was a strong trend for arthropod morphospecies richness to be greatest at the

highest level of genetic diversity, and overall community composition was driven by differences in genetic diversity in *A. breviligulata*. Interestingly, the effect of genetic diversity on arthropod community structure differed somewhat in communities containing only *A. breviligulata* (independent plots). When *A. breviligulata* was grown alone, genetic diversity did not significantly influence arthropod abundance or overall community structure. Additionally, the significant effect of genetic diversity on arthropod morphospecies richness took a slightly different form, although arthropod richness was still lowest in the presence of three populations.

Non-additive effects of genetic diversity were important in both experiments. In the crossed plots, the increases in arthropod abundance and richness with higher genetic diversity were caused by positive non-additive effects of diversity when six populations of *A. breviligulata* were present, illustrating that the arthropod response was an emergent property of diverse plant communities. Arthropod abundance was not correlated with plant biomass, so it is likely that these non-additive effects were driven by structural complexity in diverse communities. In contrast to the crossed plots, in the independent genetic diversity manipulation, the low arthropod morphospecies richness in plots with three populations was driven by a negative non-additive effect of diversity. These differential effects of genetic diversity show that the effect of genetic diversity depends on the presence of other species in the community, highlighting the importance of plant community composition for the mediation of arthropod community structure.

The ability of genetic diversity within plant species to more strongly affect arthropod communities than species diversity is surprising, but has discussed before (Crutsinger et al. 2006, Johnson et al. 2006). Crutsinger et al. (2006) found that the effect

size of genetic diversity (12 genotype populations compared to monocultures) in populations of *Solidago altissima* on arthropod diversity was nearly twice as large as the effect size of plant species diversity (16 plant species compared to monocultures) on arthropod diversity in the Cedar Creek biodiversity experiment (Siemann et al. 1998). In contrast, the only study to directly compare the effect of plant species diversity and genetic diversity in the same system found a weaker effect of plant genetic diversity than species diversity on arthropod richness. Cook-Patton et al. (*in press*) found that arthropod richness was ~35% greater in species polycultures relative to species monocultures and ~10% greater in genetic polycultures relative to genetic monocultures. In our system, we believe that genetic diversity effects were particularly strong because *A. breviligulata* is an ecosystem engineer. *Ammophila breviligulata* affects the abiotic environment by inducing sand stabilization (Olson 1958, Cheplick 2005). It also likely influences soil moisture and temperature by shading the ground and conducting water to the soil surface via evapotranspiration (Breshears et al. 1997, 1998). In addition, *A. breviligulata* makes up a significant portion of plant biomass on the sand dunes. By the end of the third growing season, *A. breviligulata* comprised 80% of the total aboveground biomass in crossed plots, providing most of the structure and resources available to arthropods.

The majority of studies investigating the effects of plant species diversity on arthropod community composition have found increasing arthropod species richness with increasing plant species richness (Siemann et al. 1998, Knops et al. 1999, Wenninger and Inouye 2008). We hypothesize that one reason we did not find an effect of species diversity on arthropod morphospecies richness because the arthropod community on the

sand dunes is dominated by predators. Theory predicts that arthropod species richness will be greater in diverse plant communities because the probability of including specialist herbivores increases (Hutchinson 1959, Strong et al. 1984). While herbivores that specialize on one plant species do exist in our system, they do not make up a significant portion of the overall arthropod community in the dunes (in our survey, 2 of the 100 morphospecies and 0.1% of the total individuals were specialists). This makes it unlikely that increases in plant species diversity will cause a significant increase in arthropod species richness.

Like other studies (Crutsinger et al. 2006, Johnson et al. 2006), we did find a significant effect of genetic diversity on arthropod communities, but this effect was dependent on the presence of other species in the community. When other species were present, genetic diversity significantly influenced arthropod abundance, morphospecies richness, and community composition. In the absence of other species, genetic diversity only affected morphospecies richness. This suggests that genetic diversity within *A. breviligulata* interacts with the other species to influence arthropods. For example, the presence of both *A. breviligulata* and other species could significantly alter habitat complexity, potentially altering interactions among plants and herbivores (Denno et al. 2005).

The significant effect of genetic diversity on arthropod community structure was driven by the responses of some of the more numerically dominant arthropod morphospecies, specifically Chironomid and Ceratopogonid midges. Chironomid and Ceratopogonid midges begin their lifecycle as aquatic larvae. As adults they emerge *en masse* to mate. Chironomid adults typically do not eat, but may eat nectar.

Ceratopogonid adults may not eat, or the females may be blood feeders. We were never bitten, so it is improbable that our morphospecies was a blood feeder. Based on their feeding strategies, it is unlikely that either midge was responding to an increase in the types of food resources at high levels of genetic diversity (Hutchinson 1959, Strong et al. 1984). Therefore, we hypothesize that the midges responded to plant structural complexity, which increased with greater *A. breviligulata* genetic diversity (Crawford, *unpublished data*). The positive response to structural complexity is also supported by the non-additive increase in arthropod abundance when six populations of *A. breviligulata* were present. Structural complexity is a property of entire communities; therefore, the response of arthropods to structural complexity cannot be predicted by their responses in monoculture. Many arthropods respond to increases in structural complexity (Halaj et al. 2000, Borges and Brown 2001, Topp et al. 2008, Pearson 2009). For example, Carabid beetle abundance was greater in areas with logging residue than on bare ground (Nitterus and Gunnarsson 2006), and spider communities respond positively to structural complexity (Halaj et al. 2000).

The greater abundance of midges in genetically diverse treatments may have cascading community-level and ecosystem-level consequences. In Iceland, up to 2500kg/ha/year of Chironomid midges are deposited along terrestrial shorelines, potentially resulting in a significant fertilization effect (Gratton et al. 2008). Our capture rate for midges was roughly 1.68g m^{-1} day^{-1} in communities with six populations of *A. breviligulata* and only half that (~0.89g m^{-1} day^{-1}) in communities with either one or three populations of *A. breviligulata.* Icelandic lakes produced approximately 5g m^{-1} day^{-1} of midge biomass (Gratton et al. 2008). While the input of midges in our system

was 1/3 of this, we suggest that the midge input represents a relatively large flux of carbon and nitrogen from lakes to the shoreline because soil in the experimental plots had no detectable nitrogen even three years after the experiment was established (Crawford, *unpublished data*). Additionally, midges are an important source of food for terrestrial consumers (Gratton et al. 2008), and may influence the abundance of terrestrial predators (Jonsson and Wardle 2009). While we found no strong correlation between midge abundance and predator abundance during the course of this study ($F_{1,61} = 1.2$, $P = 0.2770$, $r = 0.14$), the use of stable isotopes in future research could determine whether or not midges are an important resource for terrestrial arthropods in this system.

Overall, our results show that genetic diversity within a dominant plant species can have a greater effect on arthropod community structure than plant species diversity. This suggests that when managing natural systems to avoid negative consequences of biodiversity loss for higher trophic levels, it may be as important to preserve diversity in dominant plant species as it is to preserve plant species diversity. Notably, we found that the effect of *A. breviligulata* genetic diversity was strongest in the presence of other plant species. Therefore, to further improve our ability to predict the consequences of biodiversity loss, we must begin to account for the effects of multiple levels of diversity.

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3.7 TABLES

Table 3.1 Results from repeated measures mixed models testing the effects of plant diversity and time on arthropod community abundance, richness, and evenness, and PERMANOVA models testing how plant diversity influenced community composition. "Crossed GD x SD" included plots with both levels of diversity manipulated. Independent diversity manipulations are "GD only" and "SD only," and included plots where only genetic diversity or species diversity were manipulated. Bold *P*-values were significant at $P < 0.05$.

		Arthropod Community Responses											
		Abundance			Richness			Evenness			Composition		
		df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*	*df*	*Pseudo-F*	*P(perm)*
Crossed GD x SD	**GD**	2, 54	3.17	**0.0497**	2, 54	3.11	0.0525	2, 47	2.70	0.0778	2, 162	3.29	**0.0005**
	SD	2, 54	1.26	0.2921	2, 54	0.02	0.9771	2, 47	1.95	0.1530	2, 162	0.72	0.7500
	GD x SD	4, 54	0.64	0.6389	4, 54	0.58	0.6808	4, 47	1.30	0.2823	4, 162	1.00	0.4746
	Time	3, 52	57.82	**<0.0001**	3, 52	122.35	**<0.0001**	3, 48	16.55	**<0.0001**	3, 162	64.83	**0.0001**
	Time x GD	6, 68	2.72	**0.0199**	6, 68	1.10	0.3729	6, 62	1.68	0.1406	6, 162	2.87	**0.0001**
	Time x SD	6, 68	1.06	0.3932	6, 68	0.57	0.7549	6, 62	1.07	0.3876	6, 162	0.86	0.6959
	Time x GD x SD	12, 86	0.90	0.5511	12, 86	0.68	0.7648	12, 79	0.94	0.5119	12, 162	0.82	0.8354
GD Only	**GD**	2, 53	0.66	0.5194	2, 53	3.43	**0.0398**	2, 52	0.89	0.4151	2, 159	0.96	0.4864
	Time	3, 51	29.99	**<0.0001**	3, 51	71.86	**<0.0001**	3, 51	13.87	**<0.0001**	3, 159	25.56	**0.0001**
	Time x GD	6, 67	0.55	0.7708	6, 67	1.01	0.4268	6, 67	0.87	0.5193	6, 159	0.76	0.7954
SD Only	**SD**	2, 38	2.92	0.0663	2, 38	1.29	0.2874	2, 38	0.22	0.8041	2, 114	1.81	0.0686
	Time	3, 36	31.41	**<0.0001**	3, 36	31.04	**<0.0001**	3, 35	7.76	**0.0004**	3, 114	24.802	**0.0001**
	Time x SD	6, 47	1.55	0.1823	6, 47	0.44	0.8470	6, 45	2.04	0.0800	6, 114	1.14	0.2894

3.8 FIGURES

Figure 3.1 Diagram of the experimental design for the common garden. Circles with different patterns represent different populations of *Ammophila breviligulata*. Triangles with different patterns represent different plant species. In the independent plots, only one level of diversity, either genetic diversity within *A. breviligulata* or species diversity, was manipulated. In the crossed plots, both species diversity and genetic diversity within *A. breviligulata* were simultaneously manipulated. Plots were composed of ½ *A. breviligulata* and ½ other species. All plots contained 24 plants, except for monocultures, which had 12. Pitfall traps were placed near the center of each plot to collect arthropods.

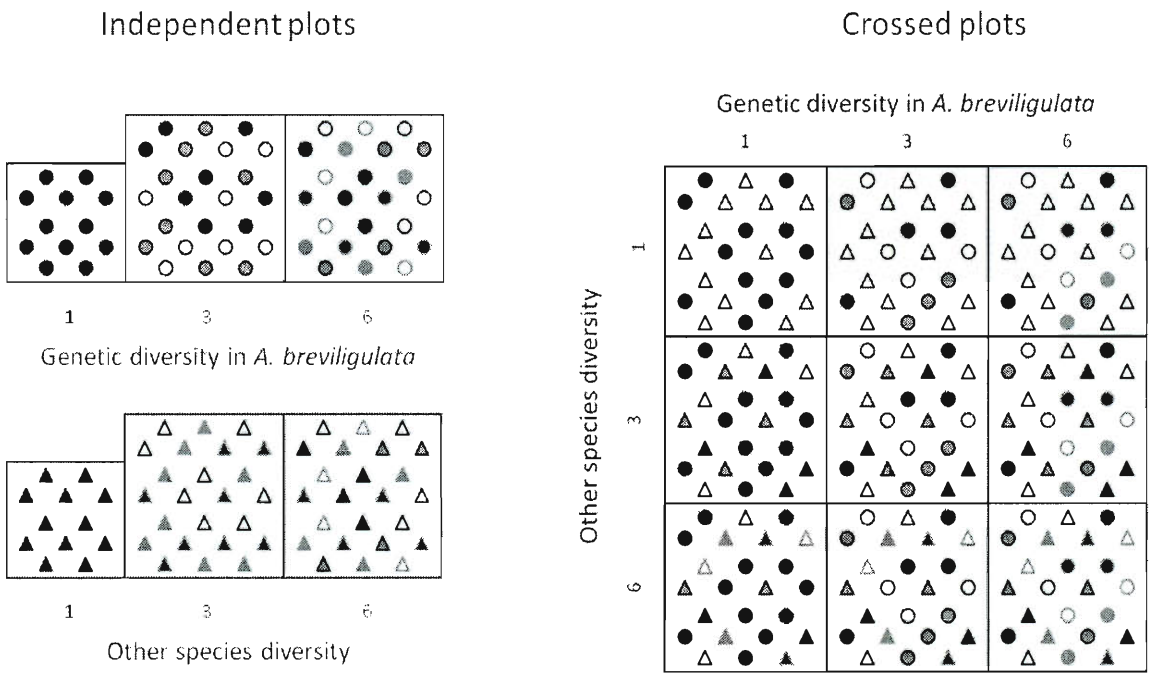


Figure 3.2 Non-metric multidimensional scaling ordination of PERMANOVA results showing that the effect of *Ammophila breviligulata* genetic diversity on arthropod community composition was dependent on time in crossed plots. Centroids of the genetic diversity treatments are graphed for each month ± s.e. The distance between symbols reflects similarity in species composition.

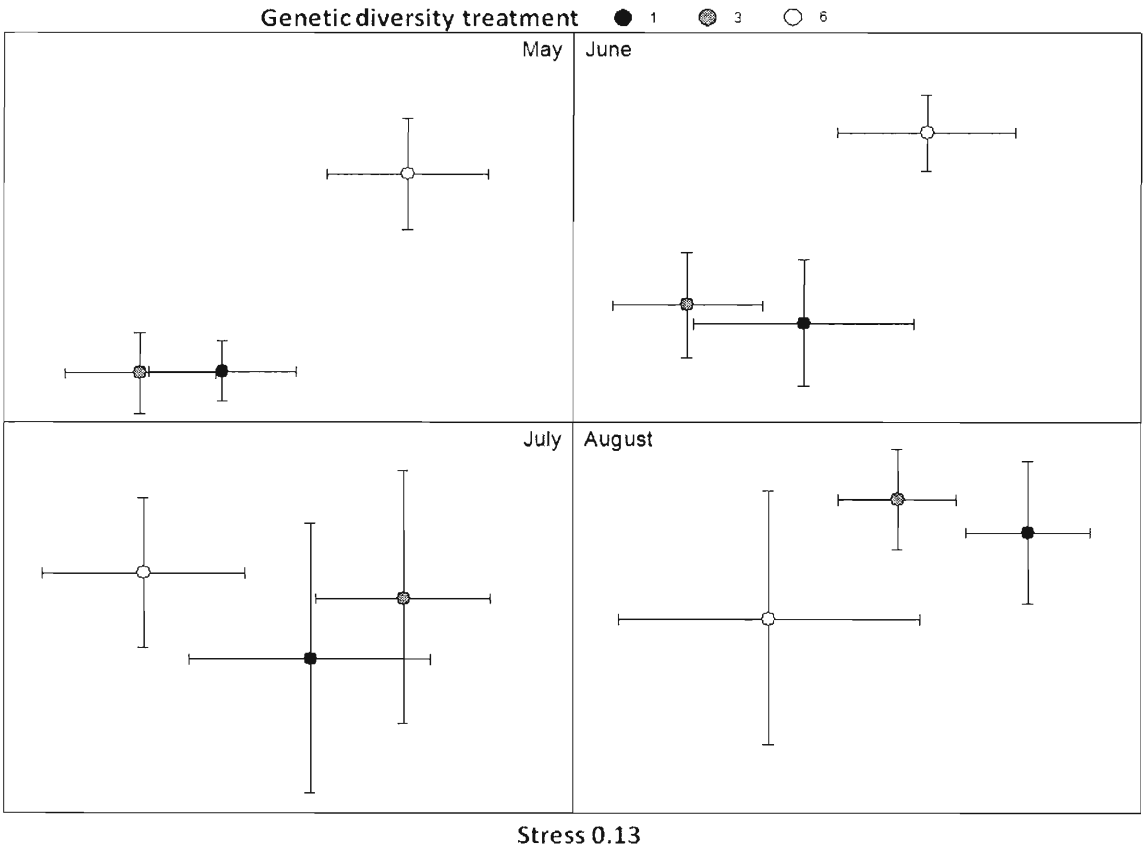


Figure 3.3 Observed treatment means and the additive expectation for the effect of genetic diversity on arthropod abundance in crossed plots. Bars show means ± s.e.. Observed treatment means that share a letter are not statistically significantly different from each other. Expected and observed pairs with a star are significantly different from each other, indicating a significant non-additive effect of diversity.

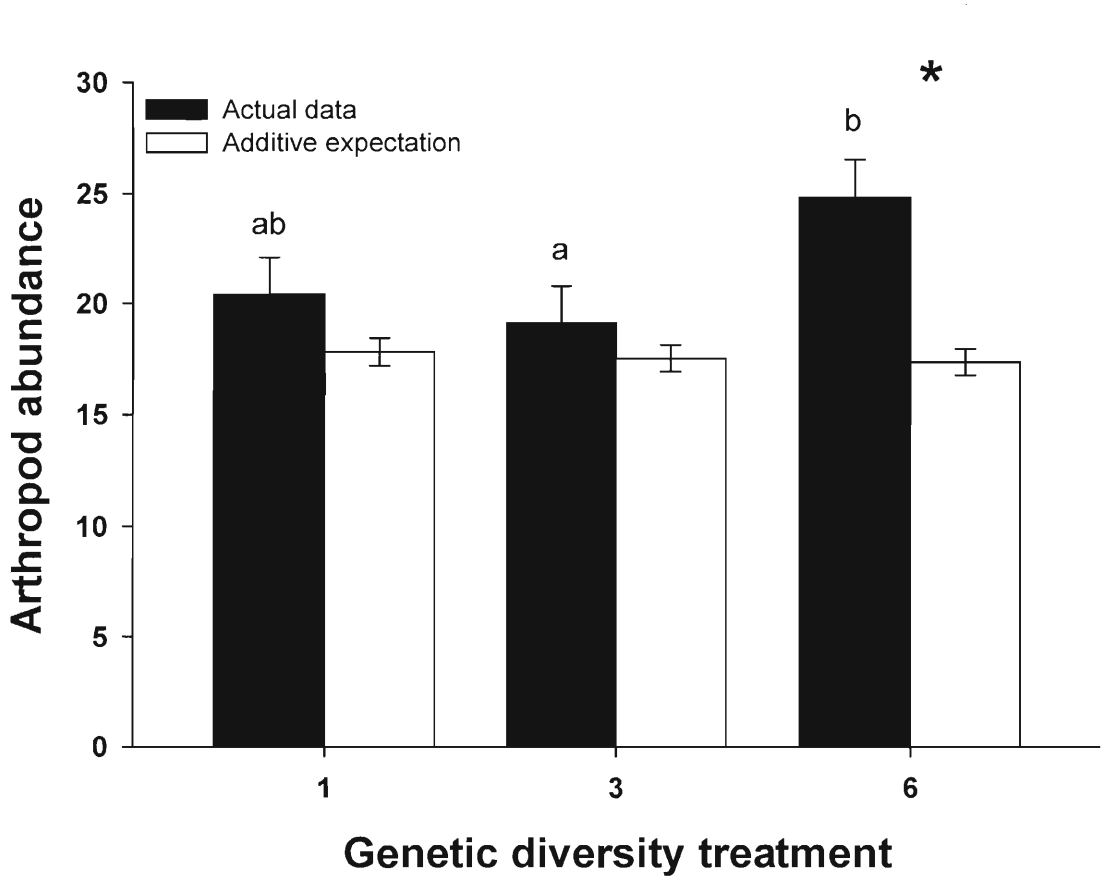
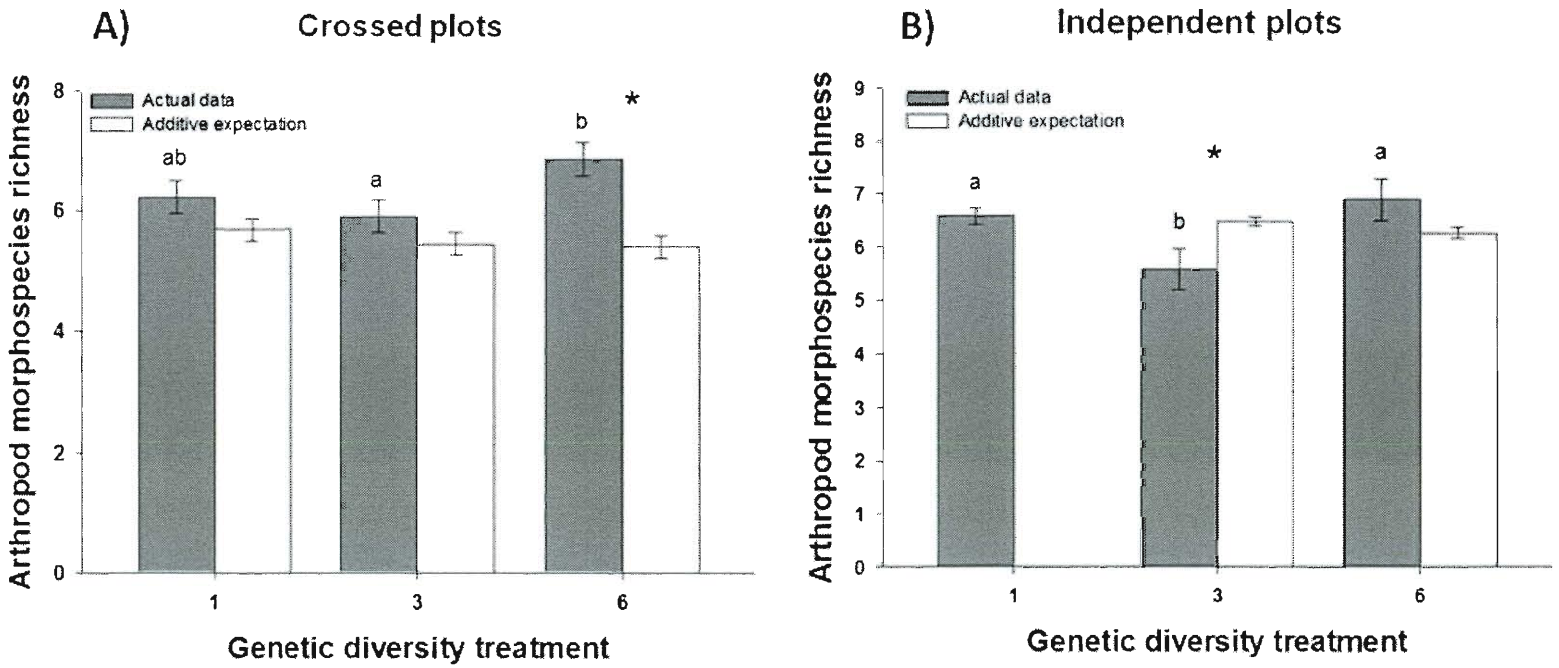


Figure 3.4 Observed treatment means and the additive expectation for the effect of genetic diversity on arthropod morphospecies richness in A) crossed plots and B) independent plots. Bars show means ± s.e. Observed treatment means that share a letter are not statistically significantly different from each other. Expected and observed pairs with a star are significantly different from each other, indicating a significant non-additive effect of diversity. For the independent plots, there is no expected value when one population of *A. breviligulata* is present (genetic diversity treatment level 1), because values to conduct the additivity analysis were drawn from those monoculture plots.



3.9 APPENDIX A

Table 3.A1 List of morphospecies collected in the experimental plots, along with their total abundances, order and family identifications, and functional group assignments.

Morphospecies Code	Order	Family	Functional Group	Total Abundance
HYM1	Hymenoptera	Formicidae	Omnivore	3324
DIP14	Diptera	Ceratopogonidae		2761
COLE1	Coleoptera	Anthicidae	Detritivore	1747
DIP1	Diptera	Chironomidae	Non-feeding	1137
AC1	Acari			922
AC2	Acari	Orbatida	Detritivore	894
HEM2	Hemiptera	Aphididae	Herbivore	451
HEM1	Hemiptera	Cercopidae	Herbivore	378
AR1	Araneae	Lycosidae	Predator	192
DIP33	Diptera	Sciaridae	Detritivore	148
DIP10	Diptera	Cecidomyiidae		132
DIP6	Diptera	Dolichopodidae	Predator	121
COLL2	Collembola	Isotomidae	Detritivore	107
DIP11	Diptera	Cecidomyiidae		97
COLE14	Coleoptera	Cicindelidae	Predator	94
LEP3	Lepidoptera	Lasiocampidae	Herbivore	78
DIP2	Diptera	Chironomidae	Non-feeding	74
HYM2	Hymenoptera	Formicidae	Omnivore	74
AR4	Araneae	Pisauridae	Predator	67
HEM4	Hemiptera	Aphidoidea	Herbivore	61
DIP9	Diptera	Chironomidae	Non-feeding	48
COLE20	Coleoptera	Histeridae	Predator	41
DIP20	Diptera	Chloropidae		39
DIP5	Diptera	Fanniidae		39
AR2	Araneae	Salticidae	Predator	34
HYM6	Hymenoptera	Braconidae	Parasitoid	30
HYM7	Hymenoptera	Signiphoridae	Parasitoid	27
HYM4	Hymenoptera	Chalcidoidea		24
HYM3	Hymenoptera	Xiphydriidae		23
DIP26	Diptera	Ephydridae	Predator	20
AR5	Araneae	Pisauridae	Predator	18
COLE2	Coleoptera	Chrysomelidae	Herbivore	15
DIP31	Diptera	Sarcophagidae		15

Table 3.A1 (continued)

Morphospecies Code	Order	Family	Functional Group	Total Abundance
AR8	Araneae	Thomisidae	Predator	15
COLE18	Coleoptera	Elateridae	Herbivore	14
OP1	Opiliones		Predator	13
COLE13	Coleoptera	Chrysomelidae	Herbivore	12
TRI2	Tricoptera	Beraeidae	Non-feeding	12
HYM19	Hymenoptera	Pompilidae	Predator	12
DIP32	Diptera	Sarcophagidae		9
DIP23	Diptera	Dixidae		9
TRI4	Tricoptera		Non-feeding	9
PSO2	Psocoptera			8
DIP24	Diptera	Drosophilidae		8
LEP4	Lepidoptera	Lasiocampidae	Herbivore	8
THY2	Thysanoptera			8
DIP8	Diptera	Empididae	Predator	8
COLE6	Coleoptera			7
DIP16	Diptera	Asteiidae		7
DIP19	Diptera	Chloropidae		7
HYM17	Hymenoptera	Cynipoidea	Parasitoid	7
MEG1	Megaloptera		Non-feeding	7
AR7	Araneae	Thomisidae	Predator	6
DIP22	Diptera	Culicidae		6
DIP3	Diptera	Empididae	Predator	6
HYM14	Hymenoptera	Crabronidae	Predator	6
HYM21	Hymenoptera	Vespidae	Predator	6
HYM22	Hymenoptera	Vespidae	Predator	6
NEU1	Neuroptera	Chrysopidae		6
DIP30	Diptera	Psychodidae		5
DIP27	Diptera	Fanniidae		5
AR3	Araneae	Lycosidae	Predator	5
COLE11	Coleoptera	Coccinellidae	Predator	5
HEM3	Hemiptera	Phymatidae	Predator	5
HYM13	Hymenoptera	Chrysididae	Predator	5
COLE3	Coleoptera	Chrysomelidae	Herbivore	4
DIP21	Diptera	Chloropidae		4
DIP4	Diptera	Therevidae		4
HEM5	Hemiptera	Phylloxeridae	Herbivore	4
TRI3	Tricoptera		Non-feeding	4
COLE12	Coleoptera	Coccinelidae	Predator	4
COLE7	Coleoptera	Staphylinidae	Predator	4

Table 3.A1 (continued)

Morphospecies Code	Order	Family	Functional Group	Total Abundance
DIP7	Diptera	Asilidae	Predator	4
HYM18	Hymenoptera	Icneumonidae	Parasitoid	4
DIP29	Diptera	Mycetophilidae		3
ISO1	Isoptera		Detritivore	3
MYR1	Myripoda (subphylum)		Detritivore	3
COLE23	Coleoptera	Scarabidae		3
COLE24	Coleoptera	Scarabidae		3
DIP34	Diptera	Sciomyzidae	Predator	3
LEP2	Lepidoptera		Herbivore	3
OR1	Orthoptera	Acrididae	Herbivore	3
THY1	Thysanoptera			3
DIP28	Diptera	Fanniidae		3
HYM5	Hymenoptera	Proctotrupoidea	Parasitoid	3
PSO3	Psocoptera			2
COLE19	Coleoptera	Elateridae	Herbivore	2
COLE21	Coleoptera	Scarabidae	Herbivore	2
DIP15	Diptera	Cecidomyiidae		2
DIP17	Diptera	Cecidomyiidae		2
DIP25	Diptera	Drosophilidae		2
LEP1	Lepidoptera		Herbivore	2
TRI1	Trichoptera		Non-feeding	2
TRI5	Trichoptera		Non-feeding	2
AR6	Araneae	Thomisidae	Predator	2
COLE25	Coleoptera	Staphylinidae	Predator	2
COLE8	Coleoptera	Carabidae	Predator	2
HEM6	Hemiptera	Rhyparochromidae	Herbivore	2
HYM10	Hymenoptera	Braconidae	Parasitoid	2
HYM16	Hymenoptera	Crabronidae	Predator	2

Table 3.A2 Average abundance of each morphospecies for each genetic diversity treatment in crossed plots.

Average Abundance in Crossed Plots			
	Genetic Diversity Treatment		
Morphospecies Code	**1**	**3**	**6**
HYM1	5.75	5.333333	4.27381
DIP14	3.52381	3.416667	7.714286
COLE1	3.154762	2.464286	2.690476
DIP1	1.869048	1.607143	2.654762
AC1	1.071429	1.738095	0.77381
AC2	0.97619	0.904762	2.333333
HEM2	0.404762	0.630952	0.630952
HEM1	0.869048	0.630952	0.404762
AR1	0.404762	0.130952	0.357143
DIP33	0.261905	0.178571	0.285714
DIP10	0.166667	0.22619	0.297619
DIP6	0.202381	0.154762	0.130952
COLL2	0.119048	0.202381	0.130952
DIP11	0.071429	0.095238	0.142857
COLE14	0.142857	0.035714	0.107143
LEP3	0.119048	0.071429	0.095238
DIP2	0.095238	0.119048	0.142857
HYM2	0.059524	0.083333	0.166667
AR4	0.047619	0.107143	0.130952
HEM4	0.035714	0.035714	0.214286
DIP9	0.059524	0.119048	0.011905
COLE20	0.011905	0.035714	0.071429
DIP20	0.071429	0.059524	0.107143
DIP5	0.095238	0.071429	0.071429
AR2	0.071429	0	0.071429
HYM6	0.035714	0.011905	0.02381
HYM7	0.035714	0	0.107143
HYM4	0.02381	0.035714	0.011905
HYM3	0.035714	0.035714	0.02381
DIP26	0.107143	0	0.011905
AR5	0	0.011905	0.059524
COLE2	0.02381	0	0.011905
DIP31	0	0.059524	0
AR8	0.02381	0.035714	0.02381

Table 3.A2 (continued)

Morphospecies Code	1	3	6
OP1	0.02381	0.011905	0.011905
COLE13	0.047619	0	0
TRI2	0	0.035714	0.047619
HYM19	0.02381	0.02381	0.035714
DIP32	0.011905	0	0
DIP23	0	0	0.02381
TRI4	0	0.035714	0.011905
PSO2	0.035714	0.011905	0
DIP24	0.011905	0.047619	0.02381
LEP4	0	0	0.02381
THY2	0	0.011905	0
DIP8	0	0	0.011905
COLE6	0.02381	0	0
DIP16	0.02381	0	0.035714
DIP19	0	0.02381	0
HYM17	0	0.035714	0
MEG1	0.035714	0.011905	0.011905
AR7	0.02381	0.011905	0
DIP22	0.02381	0.02381	0
DIP3	0.02381	0	0.02381
HYM14	0	0	0.02381
HYM21	0	0	0.02381
HYM22	0	0	0
NEU1	0	0	0
DIP30	0	0.02381	0.011905
DIP27	0	0	0.02381
AR3	0.02381	0.011905	0
COLE11	0	0	0.011905
HEM3	0	0	0
HYM13	0.011905	0	0.02381
COLE3	0	0.011905	0
DIP21	0	0.011905	0
DIP4	0	0	0
HEM5	0.02381	0	0.02381
TRI3	0	0	0
COLE12	0	0.02381	0.02381
COLE7	0	0	0.011905
DIP7	0.011905	0.011905	0
HYM18	0	0	0
DIP29	0.011905	0.011905	0.011905

Table 3.A2 (continued)

Morphospecies Code	1	3	6
ISO1	0	0.011905	0.011905
MYR1	0	0	0.02381
COLE23	0	0	0
COLE24	0.011905	0	0
DIP34	0.011905	0	0
LEP2	0	0	0
OR1	0.011905	0	0.011905
THY1	0	0.011905	0
DIP28	0	0	0
HYM5	0.011905	0	0
PSO3	0	0.011905	0
COLE19	0	0	0
COLE21	0	0	0
DIP15	0	0.02381	0
DIP17	0	0	0.011905
DIP25	0.011905	0	0
LEP1	0	0	0
TRI1	0	0	0
TRI5	0	0	0
AR6	0	0	0
COLE25	0	0	0
COLE8	0	0	0
HEM6	0	0	0.02381
HYM10	0	0	0
HYM16	0	0.011905	0.011905

Table 3.A3. Average abundance of each morphospecies for each genetic diversity treatment in the independent genetic diversity manipulation.

Average Abundance in Crossed Plots			
	Genetic Diversity Treatment		
Morphospecies Code	**1**	**3**	**6**
HYM1	6.660714	4	4.75
DIP14	1.803571	4.5	2.678571
COLE1	3.333333	2.928571	4.714286
DIP1	1.255952	1.535714	1.107143
AC1	2.154762	1.107143	1.785714
AC2	2.184524	0.464286	1.178571
HEM2	0.89881	0.892857	0.571429
HEM1	0.797619	0.607143	1.035714
AR1	0.285714	0.107143	0.464286
DIP33	0.238095	0.142857	0.392857
DIP10	0.22619	0.142857	0.178571
DIP6	0.113095	0	0.142857
COLL2	0.160714	0.142857	0.107143
DIP11	0.160714	0.071429	0.071429
COLE14	0.035714	0.035714	0.214286
LEP3	0.095238	0.142857	0.071429
DIP2	0.071429	0	0.071429
HYM2	0.107143	0.214286	0.107143
AR4	0.119048	0.035714	0.214286
HEM4	0.071429	0.035714	0.178571
DIP9	0.071429	0.035714	0
COLE20	0.095238	0.035714	0.071429
DIP20	0.017857	0.035714	0.035714
DIP5	0.041667	0.071429	0.107143
AR2	0.059524	0	0.107143
HYM6	0.071429	0.107143	0.107143
HYM7	0.047619	0.035714	0.035714
HYM4	0.059524	0.035714	0
HYM3	0.053571	0.035714	0
DIP26	0.047619	0	0
AR5	0.041667	0.035714	0.071429
COLE2	0	0	0
DIP31	0.02381	0	0
AR8	0	0	0

Table 3.A3. (continued)

Morphospecies Code	1	3	6
COLE18	0.035714	0	0
OP1	0.02381	0	0
COLE13	0.029762	0	0
TRI2	0.005952	0	0
HYM19	0.011905	0.035714	0
DIP32	0.02381	0.071429	0
DIP23	0.005952	0	0.035714
TRI4	0.017857	0	0
PSO2	0.005952	0.035714	0
DIP24	0.005952	0	0
LEP4	0.02381	0	0
THY2	0.011905	0.035714	0
DIP8	0.011905	0	0.035714
COLE6	0.005952	0	0
DIP16	0.005952	0.035714	0
DIP19	0.005952	0	0
HYM17	0	0	0
MEG1	0	0	0
AR7	0	0	0
DIP22	0.005952	0	0.035714
DIP3	0.011905	0	0
HYM14	0.011905	0	0
HYM21	0.011905	0	0
HYM22	0.011905	0.035714	0.035714
NEU1	0.005952	0	0.035714
DIP30	0.011905	0	0
DIP27	0.005952	0	0
AR3	0.011905	0	0
COLE11	0.011905	0	0
HEM3	0	0	0.035714
HYM13	0	0	0
COLE3	0.011905	0	0
DIP21	0.011905	0	0.035714
DIP4	0.011905	0	0
HEM5	0	0	0
TRI3	0.011905	0	0
COLE12	0	0	0
COLE7	0.011905	0	0
DIP7	0	0.035714	0

Table 3.A3. (continued)

Morphospecies Code	1	3	6
HYM18	0.011905	0.035714	0
DIP29	0	0	0
ISO1	0.005952	0	0
MYR1	0	0	0
COLE23	0.011905	0	0
COLE24	0.011905	0	0
DIP34	0	0	0
LEP2	0	0	0
OR1	0	0	0
THY1	0	0	0
DIP28	0.005952	0	0
HYM5	0.005952	0.035714	0
PSO3	0	0	0
COLE19	0	0.071429	0
COLE21	0	0	0.071429
DIP15	0	0	0
DIP17	0	0	0
DIP25	0	0	0
LEP1	0	0	0
TRI1	0.005952	0.035714	0
TRI5	0	0	0.071429
AR6	0	0	0
COLE25	0.005952	0	0.035714
COLE8	0	0	0
HEM6	0	0	0
HYM10	0.011905	0	0
HYM16	0	0	0

3.10 APPENDIX B

Table 3.B1 Results from repeated measures mixed models testing the effects of plant diversity and time on the abundance and richness of predators and herbivores and the ratio of herbivore abundance to predator abundance. "Crossed GD x SD" included plots with both levels of diversity manipulated. Independent diversity manipulations are "GD only" and "SD only," and included plots where only genetic diversity or species diversity were manipulated. Bold *P*-values were significant at $P < 0.05$.

		Herbivore and Predator Functional Group Responses											
		Herbivore Abundance			Herbivore Richness			Predator Abundance			Predator Richness		
		df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Crossed GD x SD	**GD**	2, 54	1.45	0.2438	2, 54	0.40	0.6717	2, 54	1.66	0.1998	2, 54	2.24	0.1164
	SD	2, 54	0.67	0.5141	2, 54	1.01	0.3714	2, 54	0.19	0.8249	2, 54	0.09	0.9105
	GD x SD	4, 54	0.30	0.8798	4, 54	0.07	0.9907	4, 54	0.26	0.8997	4, 54	0.56	0.6928
	Time	3, 52	26.43	**<0.0001**	3, 52	43.07	**<0.0001**	3, 52	39.64	**<0.0001**	3, 52	42.10	**<0.0001**
	Time x GD	6, 68	1.24	0.2990	6, 68	0.33	0.9177	6, 68	0.80	0.5725	6, 68	0.73	0.6307
	Time x SD	6, 68	0.90	0.4982	6, 68	0.46	0.8335	6, 68	1.61	0.1588	6, 68	1.38	0.2370
	Time x GD x SD	12, 86	0.99	0.4670	12, 86	1.31	0.2298	12, 86	0.79	0.6618	12, 86	1.01	0.4506
GD Only	**GD**	2, 53	0.06	0.9449	2, 53	0.18	0.8394	2, 53	2.07	0.1359	2, 53	2.32	0.1079
	Time	3, 51	27.51	**<0.0001**	3, 51	56.22	**<0.0001**	3, 51	14.85	**<0.0001**	3, 51	15.13	**<0.0001**
	Time x GD	6, 67	0.85	0.5372	6, 67	0.30	0.9351	6, 67	0.37	0.8969	6, 67	0.71	0.6429
SD Only	**SD**	2, 38	4.44	**0.0185**	2, 38	2.83	0.0714	2, 38	0.80	0.4577	2, 38	0.72	0.4945
	Time	3, 36	11.59	**<0.0001**	3, 36	11.43	**<0.0001**	3, 36	15.86	**<0.0001**	3, 36	15.59	**<0.0001**
	Time x SD	6, 47	1.55	0.1845	6, 47	1.26	0.2934	6, 47	1.35	0.2551	6, 47	1.24	0.3053

Table 3.B1 (continued)

Herbivore and Predator Functional Group Responses				
		Herbivore:Predator		
		df	***F***	***P***
Crossed GD x SD	**GD**	2, 34	1.07	0.3557
	SD	2, 31	2.51	0.0980
	GD x SD	4, 35	0.91	0.4688
	Time	3, 27	24.67	**<0.0001**
	Time x GD	6, 32	0.99	0.4485
	Time x SD	6, 32	1.49	0.2114
	Time x GD x SD	10, 36	0.50	0.8795
GD Only	**GD**	2, 36	0.11	0.8924
	Time	3, 41	8.13	**0.0002**
	Time x GD	5, 39	0.10	0.9921
SD Only	**SD**	2, 35	3.55	**0.0395**
	Time	3, 18	20.50	**<0.0001**
	Time x SD	6, 22	4.14	**0.0064**